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Gonadal Ecdysteroidogenesis in Arthropoda: Occurrence and Regulation

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Abstract

Ecdysteroids are multifunctional hormones in male and female arthropods and are stored in oocytes for use during embryogenesis. Ecdysteroid biosynthesis and its hormonal regulation are demonstrated for insect gonads, but not for the gonads of other arthropods. The Y-organ in the cephalothorax of crustaceans and the integument of ticks are sources of secreted ecdysteroids in adults, as in earlier stages, but the tissue source is not known for adults in many arthropod groups. Ecdysteroid metabolism occurs in several tissues of adult arthropods. This review summarizes the evidence for ecdysteroid biosynthesis by gonads and its metabolism in adult arthropods and considers the apparent uniqueness of ecdysteroid hormones in arthropods, given the predominance of vertebrate-type steroids in sister invertebrate groups and vertebrates.

Keywords

ovary; testes; hormone; reproduction

INTRODUCTION

A segmented exoskeleton and jointed appendages are the most characteristic features of species in the phylum Arthropoda, and this exoskeleton must be shed periodically and renewed to accommodate growth. Recent molecular phylogenetic analyses indicate that the morphologically diverse organisms included in this phylum have a monophyletic origin (151). It is divided into two sub-phyla: (*a*) Chelicerata (mites, ticks, spiders, and sea spiders) and (*b*) Mandibulata, subdivided into Myriapoda and Pancrustacea, which includes Crustacea (shrimp, crabs, and barnacles) and Hexapoda [Ectognatha (Diplura, Protura, and Collembola) and Insecta]. Furthermore, some investigators include Arthropoda in a monophyletic clade, Ecdysozoa (Bilateria, Protostomia), which encompasses Onychophora,

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DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

Tardigrada, and five worm phyla (151). A hard cuticle and periodic ecdysis are unifying characteristics of this clade.

Successful ecdysis of the exoskeleton depends on the precise timing of physiological and behavioral processes both before and after the morphogenetic event. In arthropods, these processes are hormonally regulated by an intracellular interaction of specific polyhydroxylated steroids, known as ecdysteroids, and a cascade of nuclear hormone receptors (87). For some arthropod groups, glands or tissues are the source of ecdysteroids released into the circulatory system during nymphal or larval development, but no biosynthetic source is known for many others. In turn, tissue ecdysteroid secretion is regulated by the nervous system through the release of neuropeptides or other messengers in response to internal and external cues. This tissue likely persists as a source of ecdysteroids in most arthropods, including primitive insects (bristletails and silverfish), that molt as adults. In contrast, most Chelicerata and higher insects (Pterygota) do not molt during the adult stage, and such tissues may degenerate prior to or at the adult molt. Ample evidence shows that the gonads of insects take on this role during the transition to the adult stage, but its conservation is not resolved among the other arthropod groups.

Ecdysteroidogenesis and metabolism in insects and other arthropods are covered in many reviews, and typically the focus is on glands or tissues in larval or nymphal stages, with the adult gonads only cursorily mentioned (12, 37, 80). This review examines the state of knowledge regarding gonadal ecdysteroidogenesis in Arthropoda. Reports of ecdysteroids in embryos are not included in this review, because the enzymatic alteration or biosynthesis of ecdysteroids in this stage is independent of the female. The review is divided into four main parts. First, evidence for ecdysteroidogenesis in adult gonads is summarized. Second, the ecdysteroid pathway is examined, with an emphasis on key steps in gonads. The third part covers ecdysteroid metabolism in adults, and the fourth part covers the regulation of gonadal ecdysteroidogenesis in insects.

METHODS FOR IDENTIFICATION AND QUANTIFICATION OF ECDYSTEROIDS

Different methods are used to determine whether a particular gland or tissue is a source of ecdysteroids (80). The tissue is excised for extraction and identification of ecdysteroids or for incubation to identify ecdysteroids secreted in vitro. Immunoassays are used to identify and quantify ecdysteroids, but more certain molecular identities are obtained with thin-layer chromatography, high-performance liquid chromatography, nuclear magnetic resonance spectroscopy, and mass spectral analysis. To demonstrate biosynthesis, tissues are incubated with radiolabeled precursors so that terminal ecdysteroid products can be identified. Another method is to excise the gland or tissue and then determine whether the ecdysteroids disappear in the live organism over time.

GONAD STRUCTURE AND ECDYSTEROID BIOSYNTHESIS

In most arthropod groups, adults are gonochoristic—individual males with testes and females with ovaries. The gonads develop from pole cells and epithelia to become paired

tubular organs that lie dorsally on each side of the gut in preadults and adults. The tubular organs branch into follicles in males and ovarioles in females, and the stem cells for oögonia and spermatogonia are clustered at the proximal, blind end (16). Gonads are covered with an endothelial sheet consisting of muscle fibers and other cell types. At the distal end, the organs join ducts and glands of ectodermal origin with an external opening at the end of the abdomen. In females, the oögonia mature into oocytes that are surrounded by a somatic epithelium, which forms an egg chamber. This epithelium is an important source of maternal investment proteins and other molecules, including ecdysteroids in insects that are taken up by the oocyte or circulate within the female. Ultimately, this cell layer secretes a membrane or shell (chorion) around the mature egg and dies during oviposition. Similarly, the spermatozoa show differentiation along the testicular follicle and are surrounded by an epithelium.

An overview of the literature for the identification or synthesis of ecdysteroids in the gonads or tissues of adult arthropods is presented below, and a more detailed summary is available in Supplemental Table 1 (follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>). Both are organized according to a recent classification of arthropod groups (44).

Superclass Chelicerata (Subphylum Arachnomorpha)

Molting as adults varies within this group. A definitive ecdysteroidogenic gland has not been identified for any taxon of Chelicerata, but different tissues or cell clusters are putative sources based on changes in cellular ultrastructure with molting cycles (12, 120). Oocytes typically develop asynchronously, and because they are not surrounded by an epithelium, circulating ecdysteroids likely are acquired and stored. In the sea spider *Pycnogonum littorale*, high concentrations of ecdysteroids are present in the body and in epidermal glands used for defense, but eggs contain low concentrations (57). For spiders (Arachnida: Aranaea), yolk deposition and hemolymph ecdysteroid levels are correlated (113), but the presence of ecdysteroids in ovaries has not been reported. Circulating ecdysteroids in the tarantula *Eurypelma californicum* are bound by hemocyanin, an oxygen carrier (62). In harvestmen (order Opiliones), oenocytes in appendages secrete ecdysteroids in vitro, and ecdysteroids were quantified in the hemolymph of adult scorpions (order Scorpiones) (12).

Ecdysteroids play important roles in the reproduction of male and female ticks (order Acari) (12, 104, 120). The integument is a primary source of ecdysteroids in all life stages, and radiolabeled ecdysone is converted into 20-hydroxyecdysone when injected into females. Ovaries accumulate ecdysteroids during oögenesis as hemolymph and whole-body titers rise in response to a blood meal and fertilization, but they do not secrete ecdysteroids in vitro (12, 104, 120).

Superclass Crustacea (Subphylum Mandibulata)

Most crustacean species molt as adults (130). The reproductive cycle of females may be synchronous, alternate with molting, or extend through molts. The epithelioid Y-organ in the cephalothorax produces ecdysteroids and is present in most adults (12, 45, 145). This gland

shares ultrastructural features common to steroid-producing cells of both insects and vertebrates. Oocytes are surrounded by a follicular epithelium, another possible source.

Ecdysteroids were quantified by an immunoassay for selected copepods (class Maxillopoda: subclass Copepoda) and amphipods (order Amphipoda) in different stages, sexes, and reproductive states for comparison to levels reported for other micro- and macrocrustaceans (7). No clear correlation between gravid and nongravid females and ecdysteroid level was evident for the different species, but it was noted that no Y-organ is known for copepods. In the female isopod *Armadillidium vulgare*, ovarian development and vitellogenin synthesis are inhibited by extirpation of the Y-organ, suggesting that it is the only ecdysteroid source (146). During a reproductive cycle, ovary and hemolymph levels of identified ecdysteroids show a similar rise and fall during ovarian maturation.

Regulation of reproduction by ecdysteroids has received more attention in commercially important decapods, such as crabs, crayfish, lobsters, and shrimp (class Malacostraca: order Decapoda), than in any other group of noninsect arthropods (12, 81, 145). These reviews conclude that (a) ecdysteroids are stored in oocytes, but there is no clear demonstration of secretion or correlation with hemolymph titer; (b) exogenous ecdysteroids affect yolk production, oocyte meiosis, or spermatogenesis, depending on species; and (c) ecdysteroid and associated nuclear hormone receptors are present (87). A favored hypothesis is that ecdysteroids secreted by the Y-organ are absorbed and transported by vitellogenin, which is incorporated into oocytes, where the ecdysteroids are enzymatically altered and stored for use during embryogenesis. More perplexing is the identification of sex steroids in different species (see below), but their signaling and regulatory roles are largely unknown.

Superclass Myriapoda

Ovaries of the centipede *Lithobius forficatus* (class Chilopoda) contain and secrete both ecdysone and 20-hydroxyecdysone in vitro (90), and both testes and ovaries convert the ecdysteroid precursor 5 β -ketodiol into ecdysone (22). Lymphatic strands surrounding the salivary glands may function as ecdysteroidogenic glands, based on their shared ultrastructure with prothoracic glands (PGs) of insects, immunostaining with antiecdysteroid antibodies, and secretion of ecdysteroids in vitro (133). These studies offer the best evidence that two different tissues are ecdysteroid sources in adult arthropods that molt.

Superclass Panhexapoda: Epiclass Hexapoda

This taxon includes the class Entognatha (orders Diplura, Protura, and Collembola), which are small, flightless arthropods. They molt as adults, but there are no reports on ecdysteroids in these groups. The majority of hexapod species are in the class Insecta (= Ectognatha). Most species of insects are winged and do not molt as adults. For insects, oogenesis begins in the preadult or adult female, depending on whether the female relies on larval nutrient stores for oogenesis and vitellogenesis or feeds as an adult to support these processes (3). Ovaries are classified according to the fate of oogonia, which are surrounded by a follicular epithelium. In panoistic ovarioles, oogonia become oocytes, and in meroistic ovarioles, oogonia divide into an oocyte and nurse cells that supply the oocyte with maternal macromolecules for provisioning and development. This group is further subdivided into

telotrophic or polytrophic ovarioles, based on whether the nurse cells remain in the germarium or accompany the oocyte in the follicle (egg chamber), respectively. Spermatogenesis is completed prior to the adult molt in most insect species, and the spermatozoa are stored either within testes or seminal vesicles.

The identity and biosynthesis of gonadal ecdysteroids are best characterized for insects relative to other arthropod groups (47, 48, 85). Alternative tissue and cell sources of ecdysteroids are known for insects (20, 115), so the presence of ecdysteroids within gonads is not definitive proof of biosynthesis, especially since lipoproteins and vitellogenin can transport these hormones and be taken up by tissues.

Order Zygentoma (silverfish).—The firebrat, *Thermobia domestica*, molts as an adult, and reproductive cycles in females are interspersed between molts. At the beginning of a cycle, hemolymph ecdysteroid titers rise and then fall as ovary levels increase and fall after oviposition (48). The source of ecdysteroids is not known, although a cephalic gland similar to the PGs is present in all life stages.

Subclass Pterygota.—Gonadal ecdysteroidogenesis in this arthropod group is supported by convincing evidence drawn from many studies (see Supplemental Table 1 for species and details). The first report to establish gonadal ecdysteroidogenesis was for ovaries of the mosquito *Aedes aegypti* (49). To date, ecdysteroidogenesis by ovaries is reported for species in nine orders (Dermaptera, Orthoptera, Dictyoptera, Isoptera, Hemiptera, Coleoptera, Hymenoptera, Lepidoptera, and Diptera) and ecdysteroidogenesis by testes is reported for species in four orders (Orthoptera, Hemiptera, Lepidoptera, and Diptera). Ovariectomy reduced ecdysteroid hemolymph titer in representative species of Orthoptera, Dictyoptera, Hemiptera, and Lepidoptera, thus establishing that ovaries are a primary source of ecdysteroids in vivo.

Both the ovaries and the testes of insects contain many different cell types, as do other associated tissues. The question of which cell type is ecdysteroidogenic was addressed directly in only a few studies. Preparations of follicle cells secreted ecdysteroids in vitro, whereas other cells from the ovaries of the cockroach *Nauphoeta cinerea* and the locust *Locusta migratoria* did not (39, 166). More ecdysteroids are secreted by testes of the gypsy moth, *Lymantria dispar*, in vitro than when extracted from the gland, and the sheath covering the testes is the apparent source (91). In addition, immunohistochemical staining of ecdysteroids was observed in the follicle cells of *L. migratoria* ovarioles (38) and the inner testicular sheath of *Heliothis virescens* (92).

PATHWAYS FOR ECDYSTEROID BIOSYNTHESIS

A generalized scheme for ecdysteroidogenesis is presented in Figure 1. The pathway is derived from the isolation of ecdysteroid precursors from the ovaries of *L. migratoria* (51) and from work using radiolabeled precursors with the ovaries of *L. migratoria* (26, 31, 53, 118), *Schistocerca gregaria* (25, 41, 118, 121), *Gryllus bimaculatus* (55), and *Drosophila melanogaster* (155), and testes of cotton leafworm *Spodoptera littoralis* (64) complemented with results from PGs of immature insects and Y-organs of crustaceans. The reports of

ecdysteroid precursor isolation from crustacean ovaries and metabolism of an ecdysteroid precursor to ecdysone by myriapod gonads prompted the more inclusive use of arthropod gonads in the review (22, 52, 145).

Ecdysteroidogenesis, albeit incompletely characterized, mimics vertebrate steroidogenesis in that precursor molecules shuttle between the endoplasmic reticulum (ER) and the mitochondria during processing (37, 42, 118), although the dogma suggesting the exclusivity of these two organelles has come under critical re-evaluation (80). As in vertebrate steroidogenesis, cholesterol is the precursor in arthropod ecdysteroidogenesis (37, 79, 80), but unlike vertebrates, arthropods cannot synthesize this molecule *de novo* and must acquire it from their diet (5). Carnivorous insects obtain cholesterol directly, but most phytophagous species must dealkylate plant sterols (5, 37, 42, 80, 117, 118, 147). Given the lack of *de novo* cholesterol synthesis in arthropods, cholesterol for ecdysteroidogenesis must come from either hemolymph lipoproteins or the plasma membrane. In lepidopteran insects, cholesterol absorbed by the midgut is loaded into lipoproteins and transported to peripheral tissues for internalization (15, 67). Lipoprotein delivery to the ovaries of two members in this order has been reported (66, 71, 72).

The first step of ecdysteroidogenesis is the delivery of cholesterol (1) (Figure 1) to the ER, where the first reaction is catalyzed by a cytochrome P450 enzyme (P450), 7,8-dehydrogenase (A), producing 7-dehydrocholesterol (2) (42). An oxygenase-like protein with a Rieske electron carrier domain was identified as a possible 7,8-dehydrogenase, as suggested by rescue of a *neverland* mutant (*nvd*) that was provided 7-dehydrocholesterol but not cholesterol, as well as re-establishment of normal ecdysone titers in *D. melanogaster* (165). Thus, the authors concluded that *nvd* may function at a step between cholesterol acquisition and production of 7-dehydrocholesterol (e.g., 7,8-dehydrogenase). The enzymatic activity, however, has yet to be confirmed. Transcript for the *nvd* gene was observed in ovary nurse cells of *D. melanogaster*, a possible source for ovarian ecdysteroids. Because of 7-dehydrocholesterol abundance in ecdysteroidogenic glands and the lack of trophic hormonal stimulation of its synthesis, the delivery of cholesterol to the ER and its subsequent 7,8-dehydrogenation is not considered the rate-limiting step of ecdysteroidogenesis (42). Various sterol transfer proteins, such as the diazepam-binding inhibitor, start1 and sterol-carrier protein 2 (5, 78, 128, 141, 149), may be responsible for the delivery of cholesterol to the ER or mitochondria, but none conclusively mediates this process. Control of sterol trafficking during ecdysteroidogenesis has been recently reviewed (61). The three putative sterol transfer proteins identified in insects are expressed in insect ovaries, but their expression in insect testes has not been addressed (75, 78, 128, 140). Recently, three other cholesterol transfer proteins, Niemann-Pick type C 1a (NPC1a), NPC2a, and NPC2b (29, 59, 60), have been implicated in mediating sterol transfer during ecdysteroidogenesis in *D. melanogaster* largely on the basis of mutant analysis in larval ecdysteroidogenesis. However, NPC1a expression and function in adult gonads have not been addressed. Studies on NPC1a and NPC2 demonstrate that cholesterol availability is important in the regulation of ecdysteroidogenesis, and certainly their function in arthropod gonad ecdysteroidogenesis needs to be addressed.

Following 7,8-dehydrogenation, 7-dehydrocholesterol is transferred to the mitochondria, which is considered the rate-limiting step of insect ecdysteroidogenesis (43, 156). In the inner mitochondrial membrane, a series of reactions termed the black box takes place, and various steps and precursors within this black box have been proposed (37, 42, 80, 119). Gene products of the *D. melanogaster* mutants *spook* and *spookier* have been hypothesized to conduct one of the much elusive black box reactions (107). *spook* is expressed in the follicle cells of adult ovaries (the presumed source of ecdysteroids) when ecdysteroidogenesis occurs but not during larval development, whereas its paralog, *spookier*, is expressed in larval PG but not in the follicle cells of ovaries (107). Confirmed enzymatic activity, however, awaits definitive assignment for both enzymes.

The ecdysteroid precursor following the black box modifications has eluded characterization (37, 42, 119). Involvement of a 3-oxo-⁴ intermediate has been addressed through both the metabolism of radiolabeled precursors by locust ovaries (19, 31, 116) and the metabolism of such a 3-oxo-⁴ intermediate by Y-organ preparations from the littoral crab *Carcinus maenas* (6). The latter involved a 5 β -reduction of the 3-oxo-⁴ intermediate, using an NADH-dependent cytosolic 5 β -reductase (**B**) (6). No such 5 β -reductase has been characterized from insects (37).

Ecdysteroids secreted by lepidopteran PG (42, 74) and the Y-organ of many crustaceans retain the 3-oxo group (18). The hemolymph 3-dehydroecdysone-3 β -reductase (**G**) that reduces the secreted 3-oxo-ecdysteroids is characterized for Lepidoptera (14, 74). The fact that both 5 β -diketol (**4a**) and its 3-oxo-reduced product, 5 β -ketodiol (**4b**), were isolated from and metabolically converted by insect gonads (Supplemental Table 1), and the lack of 3 β -reductase (**C**) activity in the hemolymph of nonlepidopteran insects (74), suggests that 3 β -reduction may occur within insect gonads.

Early studies on the hydroxylases catalyzing hydroxylations of either 5 β -diketol (**4a**) or 5 β -ketodiol (**4b**) (35, 68–70, 100, 118) indicated that the P450s conduct their hydroxylation reactions in a preferred sequence, C25, C22, and C2, and this finding has been corroborated (103, 157, 158). With the isolation of various terminal ecdysteroids lacking one or more of these hydroxyl groups from arthropod ovaries and eggs (54, 143, 145), the sequence of terminal hydroxylation reactions may not be steadfast. An example of such an alternative biosynthetic pathway is observed in silkworm, *Bombyx mori*, ovarian ecdysteroidogenesis (101, 105, 106, 143). However, the rates of both formation and further transformation of a reputed intermediate are critical in evaluating the overall significance of a pathway, and the possibility exists that compounds may be detectable because they are not metabolized further (116). As for the current review, the widely accepted privileged sequence (C25, C22, C2) is followed.

The P450s conducting the terminal hydroxylation reactions of ecdysteroidogenesis have been localized to the ER (25-hydroxylase, CYP306A1) and mitochondria [22-hydroxylase, CYP302A1 (**E**) and 2-hydroxylase, CYP315A1 (**F**)] (111, 158), further corroborating that ecdysteroidogenesis requires the transfer of ecdysteroid precursors between these organelles during processing (37, 69, 118). An increase in gene transcripts encoding these three P450s correlates with increases in ecdysteroid titers during both embryonic and postembryonic

development in *D. melanogaster*, *B. mori*, and *Manduca sexta* (13, 103, 110, 123, 157, 158). Gene transcripts of CYP306A1 and CYP315A1 localize to both nurse and follicle cells of *D. melanogaster* ovarioles (103, 157, 158), whereas gene transcripts for CYP302A1 were observed only within ovariole follicle cells of *D. melanogaster* (13). Homologs of CYP302A1 and CYP315A1 have been identified from the ovaries of the mosquito *Aedes aegypti*, and their transcription is highest during peak ovarian ecdysteroidogenesis following a blood meal, suggesting that this process may be modulated at the transcript level (136). Furthermore, the transcription factor FTZ-F1 regulates the expression of 25-hydroxylase and 22-hydroxylase of larval *D. melanogaster* ring glands (110), a process that is analogous to the regulation of steroidogenic P450 expression by its vertebrate homolog, steroidogenic factor 1 (108, 109).

The enzyme responsible for providing reducing equivalents to mitochondrial P450s, adrenodoxin reductase, is expressed in the nurse cells of *D. melanogaster* ovarioles (30), whereas the ovariole localization of the enzyme responsible for providing reducing equivalents to microsomal (ER) P450s, NADPH-cytochrome P450 oxidoreductase, was not addressed in its original characterization (58). It has not been determined whether either of these enzymes is expressed in testes.

The final hydroxylation reaction at C-20 is the most studied reaction of ecdysteroidogenesis and generally occurs in tissues peripheral to the original source of ecdysteroidogenesis (138). This reaction produces the most bioactive form of ecdysteroid, 20-hydroxyecdysone (8). This reaction is common to all arthropods, based on the observation that 20-hydroxylated ecdysteroids have been isolated from species throughout Arthropoda (45, 79). 20-Hydroxylase activity (**H**) has been localized to both microsomal and mitochondrial preparations, depending on the species or tissue studied (118, 138). The P450 CYP314A1 of *D. melanogaster* was recently identified as a 20-hydroxylase (**H**), and its gene transcripts were observed primarily within tissues (e.g., Malpighian tubules, fat body) peripheral to the original source of ecdysteroids, the ring glands in mitochondria (111). Gene transcripts of CYP314A1 were observed in both ovariole nurse and follicle cells, and its expression is obligatory for progression of oogenesis (111). A homolog of CYP314A1 was recently identified from the ovaries of *A. aegypti*, and in contrast to *A. aegypti* CYP302A1 and CYP315A1, gene transcript abundance of CYP314A1 did not increase in ovaries following a blood meal (136). This observation agrees with the lack of significant 20-hydroxylase activity in *A. aegypti* ovaries following a blood meal (139), and the fact that ecdysone is the major ecdysteroid secreted by ovaries (49). CYP314A1 from *M. sexta* is expressed at a low but detectable level in adult ovaries (122).

METABOLISM OF ECDYSTEROIDS

Metabolism of ecdysteroids contributes to regulation of circulating titers throughout Arthropoda (80). Ovarian ecdysteroids may have at least three functions, depending on species: (a) stimulation of meiotic reinitiation (e.g., prawn *Palaemon serratus* and locust *L. migratoria*) (84); (b) secretion into the hemolymph for promotion of developmental events in other tissues (e.g., vitellogenin production by the fat body) (47); and (c) conversion of ovarian ecdysteroids (in many insect species and other arthropods) to various conjugates

(e.g., phosphates, acylesters, or glycosides) and transfer to the newly laid eggs, where they may function as maternal, inactive storage forms of ecdysteroids for activation and function in regulating serosal membrane events and molts in early embryogenesis before differentiation of the synthesis machinery (e.g., PG) (83, 94, 121, 145). The possibility also exists that some ovarian ecdysteroid conjugates may represent inactivation waste products (76, 152).

Conjugated ecdysteroid fractions have been isolated not only from ovaries of insects, but also from ovaries of crustaceans (79, 145, 146, 161), Chelicerata (23, 120), and a myriapod (90), and from insect testes (56, 91). Most ecdysteroid conjugates identified in insect ovaries/eggs are 22-phosphates or 22-fatty acyl esters, with the latter identified also in eggs of some tick species (80, 83, 119, 120). An ecdysteroid 22-kinase from the cytosol of *B. mori* ovaries has been purified and cloned (142).

An association between early embryonic development and increase in free ecdysteroids (i.e., following hydrolysis) has been made for insects (54, 132, 143), but such a correlation has not been observed for other arthropods, such as crustaceans and acarines (94, 120, 145). Ecdysteroid phosphate phosphatase, an enzyme that releases ecdysteroids from conjugates bound to vitellin in yolk granules in *B. mori* eggs, has been cloned and its activity is correlative with both an increase in free ecdysteroid titers and embryonic development (143, 163). In a similar manner to the ecdysteroid phosphates, there is evidence suggesting that the maternal ecdysteroid fatty acyl esters undergo hydrolysis in the early stages of embryogenesis in the cricket *Acheta domesticus* (24) and the cockroach *Periplaneta americana* (137).

VERTEBRATE-TYPE STEROIDS

Steroid hormones, such as estradiol, testosterone, pregnenolone, and progesterone (herein called sex steroids), have been isolated from many arthropods (17, 81, 148). The importance of sex steroids in Crustacea is suggested by the correlation of their levels and gonad maturation, and sex steroid treatment stimulates vitellogenesis (145). In insects, their function and synthesis remain in question (129), especially because their presence may be due to ingestion or xenobiotic detoxification (148). The presence of sex steroids in arthropods is certainly intriguing, given the presumed primary hormonal role of ecdysteroids, and bears further study.

Both ecdysteroids and sex steroids occur in other invertebrate groups (Protostomia, Bilateria: coelenterates, sponges, helminthes, annelids, and mollusks) (63, 81). Biosynthesis of the two types of steroids is sufficiently different that the evolutionary conservation of their biosynthetic pathways suggests both are functionally important. Although a hormonal role for sex steroids is not established in most invertebrates, they may be important for defense, communication, and lipid and steroid metabolism (81). Many invertebrates are parasites of vertebrates, and host steroid hormones also can affect parasite development or reproduction through a conserved signaling pathway (28) (see Reference 148 for effects of host sex steroids on the rabbit flea, *Spilopsyllus cuniculi*). A more recent concern is that human

steroid drugs and pollutants acting as agonists or antagonists can disrupt the development and reproduction of diverse invertebrates in freshwater and marine environments (63).

For one species of Nematoda, which is included in Ecdysozoa, and for mollusks, compelling evidence indicates that sex steroids play a primary regulatory function. Development of the nematode *Caenorhabditis elegans* requires cholesterol (27, 34), and identified sex steroids are likely ligands for nuclear hormone receptors involved in the regulation of life span (8). The identification and function of sex steroids are best characterized for mollusks (63, 81). They are present in hemolymph and gonads and regulate sex differentiation, vitellogenesis, and immunity. The predominance of sex steroids in these two invertebrate groups stands in contrast to the ecdysteroids in arthropods, their nearest phylogenetic branch. More intriguing is the evolutionary conservation of sex steroids among protostomian invertebrates and Deuterostomia (Bilateria), including echinoderms (81) and vertebrates.

Regulation of Ecdysteroid Biosynthesis

For insects and other arthropods, ecdysteroid levels typically rise and fall over a reproductive cycle or within the adult life span. For all noninsect arthropods, accumulated evidence indicates that the gonads are not ecdysteroidogenic and that ecdysteroids originate from the Y-organ or other tissues and are incorporated into the gonads, via vitellogenin or other lipoproteins, where they may be enzymatically altered and stored. Thus, regulation of transport protein secretion is of key importance, and different peptide hormones and juvenile hormone or analogs are involved (12, 114, 120).

Ecdysteroidogenesis by insect gonads is regulated by three mechanisms: substrate availability, autocrine feedback, and peptide hormones. There are no published studies specifically addressing the first two mechanisms in gonads. Cholesterol is an essential nutrient, so its availability for ecdysteroidogenesis, relative to other cellular processes both within the insect and gonadal cells, may limit this process. Similarly, ecdysteroid intermediates may be limited inside the ecdysteroidogenic cell, depending on the activation, kinetics, or promiscuity of enzymes or transport proteins. Autocrine feedback of extracellular ecdysteroids to the cell source may act either positively to stimulate biosynthesis or negatively to reduce biosynthesis, as explored for insect PGs (131). An obvious feedback mechanism is the ecdysteroid and nuclear hormone receptor cascade through which ecdysteroids may activate or inhibit transcription of genes encoding proteins involved in their biosynthesis (114). Different transcription factors, such as *without children*, *ecdysoneless*, and *molting defective*, when genetically mutated, disrupt ecdysteroid biosynthesis in *D. melanogaster* larvae or reproduction in adults (33, 65, 102).

For several insects, but no other arthropods, identified neuropeptides and second messengers regulate gonadal ecdysteroidogenesis (21, 114). Release of regulatory peptides from neurosecretory cells likely occurs in response to external (e.g., season) and internal cues (e.g., nutrient stores or food ingestion), as integrated within the nervous system, but these mechanisms are unknown. Furthermore, gonadotropic processes are likely to be modulated by neurohormone-releasing factors and ecdysteroidostatic/oostatic peptides from the gonads or other tissues (1, 2) and by juvenile hormone (114). For example, a factor released from ovaries shortly after *A. aegypti* ingests blood stimulates the release of an “egg development

neurohormone” (89), and an oostatic factor released from ovaries with mature eggs blocks oogenesis if females take another blood meal (88). These factors have not been identified.

Hormone activation and inhibition of ecdysteroid biosynthesis are well characterized for insect PG and crustacean Y-organs (12, 37, 131, 145). The prothoracicotropic hormone (PTTH; ~30 kDa) directly stimulates the PG of immature insects through gene transcription, translation, and phosphorylation events. A peptide with the FXPRL-amide sequence also stimulates ecdysteroid release from lepidopteran PG (159), and peptides related to allatostatin B (AWQDLNSAW-amide) and myosuppressin (FLRF-amide) inhibit this process (164). Their effects on gonadal ecdysteroidogenesis are not known, with the exception of one report for allatostatin B.

Gonadotropins that Activate Ovarian Ecdysteroid Production

Early endocrine studies showed that factors from neurosecretory cells in female fly and mosquito brains regulated egg maturation (88) and ovarian ecdysteroidogenesis in vitro (see 11, 47, and references therein). Subsequently, different gonadotropins were isolated and characterized from other insects, but only a few affect ecdysteroidogenesis (21, 114). Ovary-maturing parsin (~6900 Da) from neurohemal glands in locusts was purified and structurally characterized. When injected into females, it stimulates vitellogenesis and increases hemolymph ecdysteroid titer, but no direct effect on ovarian ecdysteroidogenesis was reported. The peptide ecdysteroidogenin (~8000 Da) was partially purified from extracts of heads from adult house fly, *Musca domestica*, based on its stimulation of ovarian ecdysteroidogenesis in vitro (1). The peptide also stimulated ovarian maturation in vivo, and ecdysone, 20-hydroxyecdysone, and makisterone were produced by the peptide-activated ovaries.

Work over the past ten years revealed the structure of two endogenous neuropeptides that regulate egg maturation in blood-fed *A. aegypti* females. In independent dose-response studies, the peptides stimulate yolk deposition in decapitated, blood-fed females and activate ecdysteroidogenesis by nonoogenic ovaries in vitro. Ovary ecdysteroidogenic hormone (OEH; 9000 Da) was isolated from head extracts based on the above bioactivities (11). OEH is an ortholog of the neuroparsins first identified in locust, and gene homologs are predicted for other insects (*D. melanogaster* being an exception), arthropods, invertebrates, and vertebrates (4). The *M. domestica* ecdysteroidogenin is a likely homolog, given its similar molecular weight and bioactivity.

An insulin-like peptide (ILP) in *A. aegypti* has the same bioactivity as OEH. Eight ILPs are characterized for *A. aegypti*, and ILP3 (~6000 Da) was chosen for synthesis and testing in bioassays because of its structural similarity to bovine insulin (9). Prior work showed that mammalian insulins stimulate ecdysteroidogenesis by ovaries of *A. aegypti* and the blowfly *Phormia regina* (10, 40, 96, 124). Both OEH and ILP3 are expressed in the medial neurosecretory cells (MNCs) of the female brain—the same cells shown nearly thirty years earlier to contain an “egg development neurosecretory hormone” (88). Their regulatory interaction in vivo, however, is not resolved. Similarly, the MNCs of *P. regina* also are a source of ILPs that stimulates ovarian ecdysteroidogenesis, as does a synthetic silkworm ILP (96).

Signal Transduction Pathways Regulating Ovarian Ecdysteroidogenesis

Signal transduction pathways through which identified or putative neuropeptides regulate ovarian ecdysteroidogenesis are known for only two dipteran species. How these pathways affect the mobilization of cholesterol or the transcription, translation, and posttranslational modifications of proteins or enzymes involved in biosynthesis is not known. As observed for ovarian steroidogenesis in mammals, signal transduction pathways are complex and not easily defined (127), and a similar complexity likely awaits future investigations of ovarian ecdysteroidogenesis in insects.

cAMP-dependent pathway.—In female *A. aegypti* and *P. regina*, factors from female brains promote cAMP accumulation in ovaries, and cAMP analogs induce ovarian ecdysteroidogenesis in vitro (97, 135). These results suggest a signaling pathway exists in ovaries that is comparable to that characterized for lepidopteran PG, in which PTTH activates a G-protein-coupled receptor (GPCR)/cAMP pathway (37). In the *P. regina* studies, cAMP-stimulating factors were extracted from brain regions exclusive of the pars intercerebralis, which contains the MNC. In contrast, factors from the MNC, presumably ILPs, stimulated ovarian ecdysteroidogenesis but not cAMP levels (96).

Insulin signaling pathway.—Insulin signaling through the phosphatidylinositol 3-kinase/AKT pathway activates ovarian ecdysteroidogenesis, as shown by physiological and genetic studies (40, 96, 124, 150, 153). Involvement of this pathway was established first for *A. aegypti* ovaries through the use of mammalian insulin and pharmacological agents that activated or inhibited steps in this pathway (124) and later for *P. regina* ovaries (96). Subsequent studies showed that insulin treatment of *A. aegypti* ovaries stimulates phosphorylation of the insulin receptor homolog (125) and a downstream component, AKT (a serine/threonine kinase) (126).

The insulin receptor is highly expressed in the membranes of follicle cells surrounding oocytes in *A. aegypti* ovaries (125). These same cells are an established source of ecdysteroids in other insects. The receptor disappears from these cells over the same period (24 to 36 h post blood meal) as ovarian ecdysteroidogenesis declines (125). A recent study demonstrated binding of *A. aegypti* ILP3 to ovarian membranes and cross-linking to the insulin receptor (9), thus confirming a primary role for this pathway in the activation of ovarian ecdysteroidogenesis. Similar studies established the binding of a synthetic silkworm ILP to putative insulin receptors expressed on a lepidopteran ovarian cell line (32). A different approach showed that genetic mutations blocking expression of the insulin receptor in *D. melanogaster* result in lower ecdysteroid production by ovaries (150, 153). In mammals, insulin and related peptides affect many processes associated with reproduction, including stimulation of steroidogenesis (112, 134, 162). Taken together, these studies indicate that the insulin signaling pathway is involved in the activation of a key reproductive process common to insects and mammals.

Inhibition of Ovarian Ecdysteroidogenesis

Peptide hormones and other molecules likely inhibit ovarian ecdysteroidogenesis, as shown for ecdysteroidogenesis by PG. Allatostatin B, which inhibits PG activity, also inhibited

ecdysteroidogenesis by ovaries of the cricket *G. bimaculatus* (95). Studies of ecdysteroidogenesis by *P. regina* ovaries showed that calcium is inhibitory by its activation of a calcium-calmodulin phosphodiesterase I that degrades cAMP (98). Another potent inhibitor was cGMP (99). Because brain extracts had no inhibitory activity on ovarian ecdysteroidogenesis, the authors suggest that a native inhibitory factor may originate in an abdominal tissue (99), as reported for an ecdysteroidostatin in *M. domestica* (2). This mechanism may also be conserved within arthropods. The molt-inhibiting hormone of crustaceans directly inhibits ecdysteroidogenesis by the Y-organ, as does cGMP (12). Future studies are needed to establish a role for the GPCR/cGMP pathway in the regulation of ovarian ecdysteroidogenesis.

Regulation of Ecdysteroidogenesis in Insect Testes

Factors extracted from the pupal brains of *L. dispar* stimulate larval and pupal testes to produce ecdysteroids in vitro, and one such peptide, testis ecdysiotropin (TE), was isolated and structurally characterized (2.5 kDa) (93). It has no sequence similarity to any known peptide hormone. TE-like material occurs in the brain MNC and cells in the pupal ventral nervous system. A synthetic form activates ecdysteroidogenesis by the testes of different stages of *L. dispar* (93) and the kissing bug *Rhodnius prolixus* (154). Subsequent studies using specific activators and inhibitors showed that the regulation of ecdysteroidogenesis in *L. dispar* testes involves different GPCR signaling pathways (93).

SIGNIFICANCE AND FUTURE DIRECTIONS

Whether arthropods molt or do not molt as adults, ecdysteroids in circulation or incorporated into gonads have important effects on reproduction. They promote a range of physiological processes, including yolk protein production, growth of reproductive tissues, meiotic reinitiation, gametogenesis, and embryogenesis (12, 48, 84, 86, 94, 145). The genomic action of ecdysteroids is through a conserved pathway of nuclear hormone receptors, but sex steroids in mammals also are ligands for GPCRs that activate diverse signal pathways involved in short-term nongenomic responses (160). Homolog steroid GPCRs likely exist in arthropods, and indeed a vertebrate β -adrenergic receptor homolog characterized in *D. melanogaster* binds dopamine and ecdysteroids and activates a mitogen-activated protein kinase pathway (144).

Although the tissues or glands that produce ecdysteroids have yet to be identified for many arthropod groups, evidence for ecdysteroids in whole adults or their gonads is substantial for Arthropoda. Ecdysteroidogenesis by the Y-organ in crustaceans or the integument in ticks persists in adults, but in higher insects the gonads assume this role, as the PGs deteriorate in the adults of most groups. The PGs, however, persist in adult *L. migratoria* (82) and a few other insect groups.

The apparent source of ecdysteroids in insect gonads is an epithelial layer surrounding the oocyte or testes. Evidence for testes ecdysteroidogenesis in insects and other arthropods is not robust, and other tissues associated with the male reproductive tract need to be examined. Our understanding of the evolutionary biology underlying the transition of ecdysteroidogenic tissues in larval to adult arthropods has not advanced since it was

reviewed in the late 1980s. A clue may be found in the observations that ecdysteroidogenesis and expression of ecdysteroid P450s occurs not only in PG and gonads, but also in the integument and other tissues of ticks and insects (20, 120, 136). Whereas low levels of ecdysteroid secretion may have autocrine homeostatic functions in such tissues, acquisition of the capacity for gonads to be the primary source requires regulatory mechanisms to govern secretion in response to neurohormones. These messengers allow the nervous system to coordinate reproduction with appropriate internal and external cues or with molting (if an adult trait).

Another clue may be that ecdysteroids are transported and sequestered along with yolk proteins in the oocytes of all arthropods, primarily for embryonic development. Thus, an equally important role for the endocrine system is to mobilize these proteins at appropriate times. In most arthropod females, ecdysteroids from a nongonadal tissue regulate yolk protein secretion in the heptapancreas or fat body–like tissue, whereas in female insects, the follicular epithelium surrounding the oocyte is ecdysteroidogenic. But only in Diptera do ecdysteroids stimulate the fat body to secrete yolk proteins. In all other insects, juvenile hormone stimulates this process (50, 114). Notably, the *copora allata*, which secretes juvenile hormone, persists in all life stages of insects.

Surprisingly, the dynamics of ecdysteroid secretion by ovarian follicle cells or testes sheaths have not been investigated for any insect since the initial studies of such cells in a single locust and roach species and sheaths in a few lepidopterans in the 1970s and 1980s. Comparative studies are needed for key groups within each arthropod group to examine the contribution of ecdysteroids from other tissues/glands and gonads to better understand their relative contribution to hemolymph titer and egg ecdysteroids. Defining this transition in ecdysteroidogenic tissues will lead to a greater understanding of the phylogeny of Arthropoda and other invertebrates, but it will depend on the combination of molecular and cellular studies and classic endocrine methods of identification and quantification of ecdysteroids.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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SUMMARY POINTS

1. The gonads are the primary source of ecdysteroid hormones in adult insects, but for all other arthropods, there is little evidence that the gonads produce ecdysteroids. The Y-organ in the cephalothorax of crustaceans and the integument of ticks are the source of ecdysteroids in the adult stage.
2. In all arthropod groups, ecdysteroids are stored in oocytes of females for use during embryogenesis, and ecdysteroid uptake likely occurs via yolk proteins.
3. Ecdysteroid metabolism occurs in the hemolymph and other tissues of adult arthropods.
4. Vertebrate-type steroids are identified in a few arthropod groups, but their biosynthesis, metabolism, and function are mostly unknown.
5. Ecdysteroid biosynthesis in ovaries of dipteran species is stimulated by different neuropeptides, which activate either a GPCR/cAMP or an insulin signaling pathway. This process is inhibited through a GPCR/cGMP pathway.
6. Testes ecdysteroid production in lepidopterans is stimulated by a neuropeptide, which is structurally unrelated to any known peptide hormone, and differentially regulated through GPCR pathways.

FUTURE ISSUES

1. Ecdysteroid biosynthesis by gonads should be investigated in detail and compared among different insect and arthropod groups to determine the extent of its conservation both within these groups and in the phylum.
2. When gonadal ecdysteroidogenesis is demonstrated for an arthropod, its hormonal regulation requires further exploration to elucidate conserved or unique signaling pathways. Where such pathways intervene in the pathway of ecdysteroid synthesis or secretion also must be established.
3. More studies of noninsect arthropod adults are needed to understand the biosynthesis, metabolism, and function of vertebrate-type steroids, so that the conservation of these steroids can be placed in the context of arthropod and invertebrate evolution.

Monophyletic clade:

a taxonomic group consisting of a single common ancestor and all the descendants of that ancestor

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Ecdysteroids:

steroidal hormones that act as molting hormones in arthropods; also occur in many species of plants

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Ecdysteroidogenesis:

ecdysteroid synthesis, and herein also to include production where de novo synthesis has not been confirmed

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Follicular epithelium:

membrane covering the follicular cells in the ovary

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Vitellogenin synthesis (or vitellogenesis):
synthesis of yolk protein

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Prothoracic gland (PG):

the source of ecdysteroids in larval/nymphal insects

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Germarium:

region where the germ cells reside

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Black box:

a series of uncharacterized reactions

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CYP/P450:

cytochrome P450 enzymes, a superfamily of hemoproteins

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Autocrine:

signaling that occurs when a cell secretes a hormone that binds to receptors on the same cell and leads to changes in the cell

PTTH:

prothoracicotropic hormone

Neurohemal glands:

glands or clusters of axon terminals from neurosecretory cells where neuropeptides are released for circulation

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MNC:

medial neurosecretory cell

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GPCR/cAMP:

G protein–coupled receptor signal pathway that elevates intracellular cAMP concentrations

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AKT:

a subfamily of AGC serine/threonine protein kinases, also called protein kinase B, involved in intracellular signaling pathways

GPCR/cGMP:

G protein–coupled receptor signal pathway that elevates intracellular cGMP concentrations

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Figure 1.

A generalized pathway for ecdysteroid biosynthesis. Cholesterol (**1**) is converted to 7-dehydrocholesterol (**2**) by the microsomal cytochrome P450, 7,8-dehydrogenase (**A**). 7-Dehydrocholesterol (**2**) is then transported to the mitochondria and goes through a series of "black box" reactions in which it is converted to the hypothetical 3-oxo-⁴ intermediate, 14 α -hydroxy-cholesta-4,7-diene-3,6-dione (**3**). 14 α -Hydroxy-cholesta-4,7-diene-3,6-dione (**3**) is converted to 5 β -diketol (**4a**) by the cytosolic enzyme 5 β -reductase (**B**). Two pathways may now occur from this point forward: 5 β -diketol (**4a**) moves forward to the first of the terminal hydroxylation reactions or it is converted to 5 β -ketodiol (**4b**) by a yet unidentified 3 β -reductase (**C**). Both ecdysteroid precursors are converted by the microsomal cytochrome P450, 25-hydroxylase (CYP306A1) (**D**), yielding 2,22-dideoxy-3-dehydroecdysone (**5a**) for 5 β -diketol (**4a**) and 2,22-dideoxyecdysone (**5b**) for 5 β -ketodiol (**4b**). Both ecdysteroid precursors are then transported back to the mitochondria, where they are converted by the mitochondrial P450, 22-hydroxylase (CYP302A1) (**E**) to 2-deoxy-3-dehydroecdysone (**6a**) for 2,22-dideoxy-3-dehydroecdysone (**5a**) and 2-deoxyecdysone (**6b**) for 2,22-dideoxyecdysone (**5b**). Both ecdysteroid precursors are converted by the mitochondrial P450, 2-hydroxylase (CYP315A1) (**F**) to 3-dehydroecdysone (**7a**) for 2-deoxy-3-dehydroecdysone (**6a**) and ecdysone (**7b**) for 2-deoxyecdysone (**6b**). Both ecdysteroids are then released into the cytosol, where 3-dehydroecdysone (**7a**) is converted to ecdysone (**7b**) by 3-dehydroecdysone 3 β -reductase (**G**). Ecdysone (**7b**) is then secreted by the ecdysial

tissue and converted to 20-hydroxyecdysone (**8**) by the mitochondrial P450, 20-hydroxylase (CYP314A1) (**H**) within certain peripheral tissues.

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