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Review

Sesquiterpene action, and morphogenetic signaling through the ortholog of retinoid X receptor, in higher Diptera

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ABSTRACT

Morphogenetic signaling by small terpenoid hormones is a common feature of both vertebrate and invertebrate development. Most attention on insect developmental signaling by small terpenoids has focused on signaling by juvenile hormone through bHLH-PAS proteins (e.g., the MET protein), especially as that signaling axis intersects with ecdysteroid action through the receptor EcR. However, a series of endocrine and pharmacological studies on pupariation in cyclorrhaphous Diptera have remained persistently refractory to explanation with the above two-axis model. Recently, the terpenoid compound methyl farnesoate has been physicochemically demonstrated to exist in circulation at physiological concentrations, in several mecopterid orders, including Diptera. In addition, it has also been recently demonstrated that the receptor to which methyl farnesoate binds with nanomolar affinity (ultraspiracle, an ortholog of retinoid X receptor) requires a functioning ligand binding pocket to sustain the morphogenetic transition to puparium formation. This review evaluates endocrine and pharmacological evidence for developmental pathways reached by methyl farnesoate action, and assesses the participation of the retinoid X receptor ligand pocket in signal transduction to those developmental endpoints.

1. Introduction

The classic model of endocrine orchestration of insect molting and metamorphosis is that it is primarily regulated by the steroid 20-hydroxy ecdysone (20E) and the sesquiterpene JH (Gilbert et al., 2000; Jindra et al., 2013; Fig. 1B here). 20E binds with a nuclear hormone receptor (EcR) that appears to be an insect ortholog of vertebrate FXR or LXR (Bergot et al., 1981; King-Jones and Thummel, 2005). The vertebrate receptor FXR forms a heterodimer with the retinoid X receptor (RXR, Lefebvre et al., 2010) to form a functional complex that is responsive to endogenous FXR ligand. The insect receptor EcR forms a heterodimer with an ortholog of RXR (named "USP" in mecopterid orders that include Diptera), to form the functional ecdysteroid receptor (Henrich et al., 2000). There is evidence that a JH receptor Methoprene-tolerant (MET) may physically bind with EcR/USP during ecdysone-driven larval to pupal metamorphosis (Bitra and Palli, 2009; Guo et al., 2012). With the intersection 20E/EcR and JH/MET axes, we have the molecular implementation of the classic endocrine two-hormone model of insect metamorphosis. But is it as simple as this? Is something missing?

The classic two-hormone axis of 20E-EcR/JH-MET provides an explanation of the maturation of adult structures of the genetic model *Drosophila melanogaster* (hereafter *Drosophila*) (Riddiford, 2012) and other insects (Konopova et al., 2011). However, a body of endocrine and pharmacological data on other developmentally parallel morphogenetic events, such as higher dipteran puparium formation, have not been effectively explained by this classic model (Staal, 1975; Riddiford and Ashburner, 1991; Jindra et al., 2013). Recently, physiological levels of circulating methyl farnesoate have been measured in *Drosophila* larvae (Jones et al., 2013). In addition, genetic manipulations have established that *Drosophila* USP, which exerts a nanomolar affinity for methyl farnesoate (Jones et al., 2006), requires a functioning ligand binding pocket in order to support a formation of the puparium at the end of larval development (Jones et al., 2013). In this review, we assess how recent advances in sesquiterpenoid chemistry couple with discoveries in insect nuclear receptor biochemistry and molecular genetics of metamorphosis, to enable a more comprehensive explanation of the array

Abbreviations: RXR, retinoid X receptor; EcR, ecdysone receptor; USP, ultraspiracle; FXR, farnesoid X receptor; LXR, liver X receptor; JH, juvenile hormone; Met, Methoprene tolerant; 20E, 20-hydroxy ecdysone; PTG, prothoracic gland; MDCF, methyl dichlorofarnesoate; MCFs, chlorinated methyl farnesoates; JH, juvenile hormone; bHLH-PAS, basic helix loop helix-per-arnt-sim domain; RA, retinoic acid.

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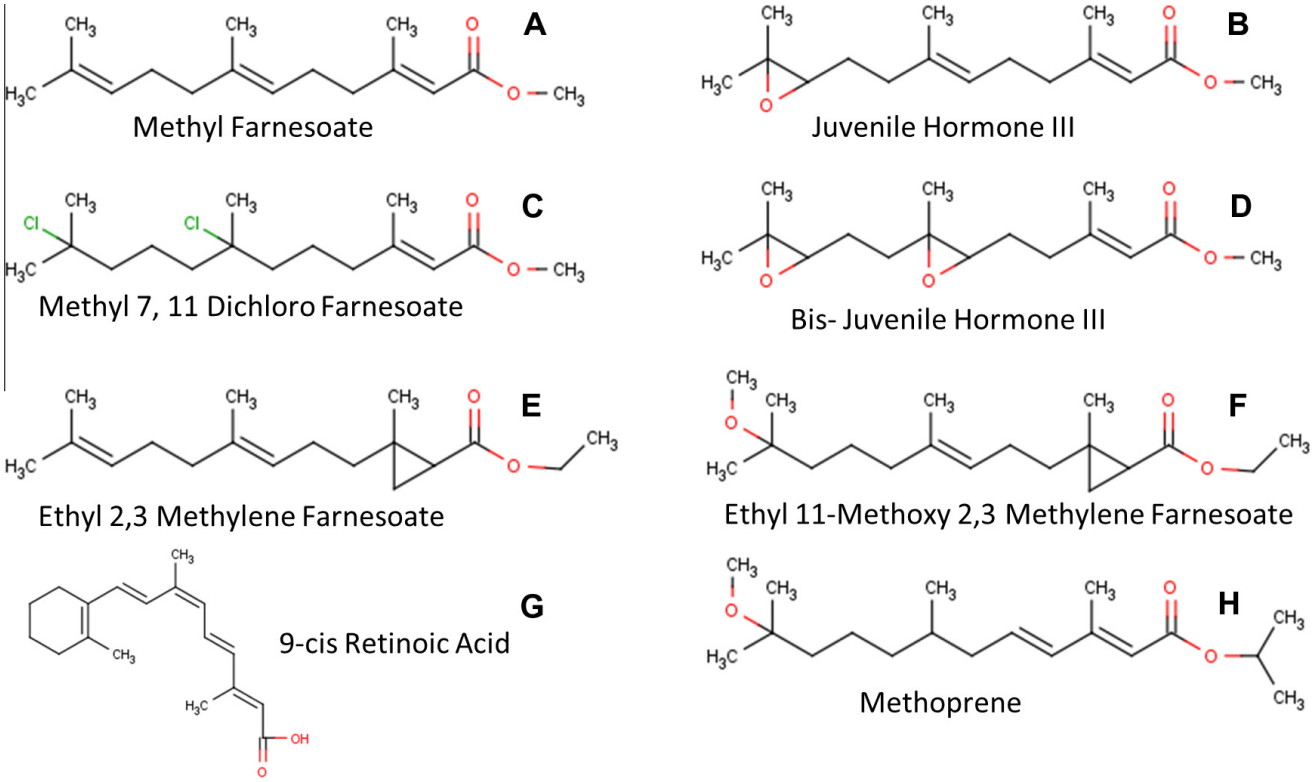

Fig. 1. Structures of natural and synthetic terpenoids as potential ligands for vertebrate RXR and mecopterid USP.

of morphogenetic phenomena occurring at the larval to pupal transition.

2. Advances in physicochemical detection of endogenous diterpenoid and sesquiterpenoid ligands

Conspicuously absent from many models of the action of vertebrate RXR, and from most models of action of mecopterid USP, is a nanomolar affinity endogenous ligand acting through RXR or USP. Indeed, there has been some consideration as to whether vertebrate or mecopterid RXR/USP even has a nanomolar affinity endogenous terpenoid or other ligand (Wolf, 2006; Markov and Laudet, 2011). However, physicochemical advances in analysis of biological samples have recently enabled the definitive measurement of physiological concentrations of endogenous terpenoids that have nanomolar affinity for binding with RXR or USP.

2.1. Detection of endogenous activating and antagonistic diterpenoid ligands of vertebrate RXR

In vertebrates, nanomolar concentrations of the diterpene 9-*cis* retinoic acid (an RXR activator, Fig. 1G) have recently been reported for the pancreas (Kane, 2012). The results of genetic interference with RXR function (Miyazaki et al., 2010), as well as pharmacological disruption of RXR action (Pérez et al., 2012), are all suggestive of the existence of a vertebrate 9-*cis* RA/RXR hormone/receptor axis in the pancreas (Kane, 2012). In addition, β-apo-13-carotenone, which has the same nanomolar affinity for RXR as does 9-*cis* RA, has recently been measured in serum at the same concentration as 9-*cis* RA (Kane, 2012). However, β-apo-13-carotenone is an RXR *antagonist* that blocks the action of agonist 9-*cis* RA at the RXR ligand binding pocket. Hence, no presumption can be made that natural, nanomolar affinity ligands for RXR (or USP, below) will necessarily be activators (Harrison et al., 2012; Eroglu et al., 2012; Sayin et al., 2013).

2.2. Detection of circulating methyl farnesoate in larval insects

Over the years bioassays, radioimmune assays, radiochemistry and various chemical methods and derivatizations have been used to measure juvenile hormone levels, including employment of both HPLC and GC modes of chromatographic separation, and coupled with various detectors for detection of various analytes (Gilbert and Schneiderman, 1960; Pratt and Tobe, 1974; Baehr et al., 1976; Bergot et al., 1981; Mauchamp et al., 1979; Richard et al., 1989; Cusson et al., 1990; Rivera-Perez et al., 2012). Our approach to identification of sesquiterpene esters from hemolymph of insects has been to use gas chromatography employing capillary columns to separate underivatized components of extracts. Coupling of capillary GC columns with mass spectroscopy (GC–MS) allows for identification and quantification methyl farnesoate, and positional and geometric isomers of its epoxidized relatives, the juvenile hormones (Fig. 1; Teal et al., 2000; Jones et al., 2013). By the above method, quantitative determination of methyl farnesoids from blood is possible on *single* larvae of *Drosophila*. These advances have enabled the larger and more complex experimental designs to discern the role in *Drosophila* of various methyl farnesoids in morphogenetic phenomena.

3. Cyclorrhaphous corpora allata secretions and larval development

The ring gland of higher (cyclorrhaphous) Diptera is a composite gland, composed of the prothoracic gland, the fused pair of corpora allata and the corpora cardiaca. Many endocrine and biochemical studies have established that the prothoracic gland portion secretes ecdysone, and the corpora allatal cells secrete