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Review

Control of ecdysteroidogenesis in prothoracic glands of insects: A review

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ABSTRACT

The very first step in the study of the endocrine control of insect molting was taken in 1922. Stefan Kopec characterized a factor in the brain of the gypsy moth, *Lymantria dispar* which appeared to be essential for metamorphosis. This factor was later identified as the neuropeptide prothoracicotropic hormone (PTTH), the first discovery of a series of factors involved in the regulation of ecdysteroid biosynthesis in insects. It is now accepted that PTTH is the most important regulator of prothoracic gland (PG) ecdysteroidogenesis. The periodic increases in ecdysteroid titer necessary for insect development can basically be explained by the episodic activation of the PGs by PTTH. However, since the characterization of the prothoracicostatic hormone (PTSH), it has become clear that in addition to 'tropic factors', also 'static factors', which are responsible for the 'fine-tuning' of the hemolymph ecdysteroid titer, are at play. Many of these regulatory factors are peptides originating from the brain, but also other, extracerebral factors both of peptidic and non-peptidic nature are able to affect PG ecdysteroidogenesis, such as the 'classic' insect hormones, juvenile hormone (JH) and the molting hormone (20E) itself. The complex secretory pattern of ecdysteroids as observed in vivo is the result of the delicate balance and interplay between these ecdysiotropic and ecdysiostatic factors.

Contents

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1. Introduction

The molting hormone, ecdysone (E) was first isolated in 1954 by Butenandt and Karlson (as reviewed by Karlson [60]). It was long thought to only be produced by the prothoracic glands (PGs) (or ring glands in Diptera) in immature insect stages, where it appeared to be essential for molting and metamorphosis. However, it is now well established that other tissues besides the PGs can perform ecdysteroid biosynthesis and that the hormone can play other major roles in the life of insects besides molting and metamorphosis. In adult females, the ovaries are the primary source of ecdysteroids, where they influence reproduction and are incorporated as conjugates into the eggs for future embryonic development. In late larval and adult males, testes also appear to be capable of producing the hormone. However, not much attention has been paid to unraveling the biological role of these testicular ecdysteroids. Moreover, oenocytes and epidermis have been proposed as alternative sites for ecdysteroid production (as reviewed by Delbecque et al. [29]; Lafont et al. [71]). Recently a lot of research has been done regarding the genes involved in the biosynthetic pathway of ecdysteroids. Insects cannot synthesize steroids from simple precursor molecules but need to take up cholesterol from their diet. A breakthrough in ecdysteroidogenesis research was the discovery of the Halloween genes. The cytochrome P450 enzymes encoded by these genes (*spook*, *spo*; *phantom*, *phm*; *disembodied*, *dib*; *shadow*, *sad* and *shade*, *shd*) catalyze a series of hydroxylation steps resulting in the active molting hormone 20-hydroxyecdysone (20E). Thanks to these characterized Halloween genes and other important genes in the biosynthetic pathway, molecular tools are now available to differentiate the primary sources (*i.e.* tissues that are capable of de novo synthesis of ecdysteroids from cholesterol) from the secondary sources (*i.e.* tissues that can release ecdysteroids after hydroxylation of late intermediates and therefore do not contain the necessary set of enzymes required for de novo synthesis) [18,90,92,118,128,144]. It is now clear that during immature insect stages, the PGs are the primary source of E, which in its turn is hydroxylated to its active form 20E in tissues peripheral to the PGs. These glands undergo apoptosis after the insect molts into the adult form, at which point ecdysteroid synthesis is taken over by the reproductive tissues. Also embryos appear to be able to synthesize ecdysteroids de novo [78]. Transcript levels of the ecdysteroidogenic enzymes encoded by the Halloween genes are critical determinants of basal PG activity (steroidogenic activity of the PG in the absence of prothoracicotropic or prothoracicostatic factors, which can be measured in vitro). Regulators of P450 gene expression are thus key players in the regulation of insect development.

In this review we will focus on the wide variety of factors influencing ecdysteroidogenesis performed by the PGs. In addition to (neuro)peptides that are released into the hemolymph and brought to the PGs, we will also focus on transport by direct innervation of PGs by peptidergic nerves. Finally we give a critical overview on our current knowledge of the action and (feedback) influence of juvenile hormone (JH) and ecdysteroids in the PGs.

Before we begin, a clarification should be made regarding the term 'prothoracicotropic hormone' or 'PTTH' and 'prothoracicostatic hormone' or 'PTSH', since this terminology may lead to some confusion. At least two different PTTHs, 'big PTTH' and 'small PTTH' are mentioned in the literature [69]. 'Big PTTH' is currently considered as the *true* PTTH as will be described in the following paragraph. 'Small PTTH' has been used to indicate smaller (MW $<$ 10 kDa) neuropeptides with tropic activity on the PGs, although these molecules are structurally not related to big PTTH (except maybe *Manduca* small PTTH). In fact, since other factors besides big and small PTTH have been demonstrated to stimulate ecdysteroidogenesis in the PGs, the term 'prothoracicotropic hormone' in the less recent literature may cover in principle *any* of these factors with tropic activity on the PGs. The same can be said regarding inhibitory factors for the term 'prothoracicostatic hormone' (PTSH).

2. PTTH–prothoracicotropic hormone

Kopec carried out the pioneering work on this neuropeptide in 1922 [70]. He provided evidence that the brain of the caterpillar, *L. dispar*, secretes a factor that can control molting and metamorphosis. The following research on this protein was primarily focused on two lepidopteran species, the silkworm, *Bombyx mori* and the tobacco hornworm, *Manduca sexta*. Many efforts were made to characterize and purify this hormone from insects, but it was not until 1991 that Kataoka et al. [61] fully determined the primary structure of the PTTH from several million silkworm brains. As shown in Table 1, *Bombyx*-PTTH is first synthesized as a 224-amino acid polypeptide precursor containing three proteolytic cleavage signals. After cleavage, it results in a 109 amino acid monomer to be assembled into a glycosylated homodimeric structure with three intramolecular disulfide bonds and a single intermolecular cysteine–cysteine bond. The folding controlled by these bonds determines the molecular structure of the protein and appears to be a prerequisite for biological activity [40,61,62]. PTTH is now cloned and purified from several other lepidopteran species such as *Antheraea pernyi*, *Samia cynthia* and *Hyalophora cecropia* (as reviewed by Rybczynski [97]) [105,109] and more recently molecular characterization yielded putative sequences of *M. sexta*, *Helicoverpa zea*, *Heliothis virescens*, *Helicoverpa armigera* and *Spodoptera exigua* PTTH [112,132,135–137]. Pairwise comparisons between amino acid sequences reveal little sequence homology, yet certain key structural features such as the presence of seven cysteine residues, a glycosylation site and the location of several hydrophobic regions are conserved.

It is now well established that two large lateral neurosecretory cells (NSCs) in the lateral region of each hemisphere of the insect brain synthesize PTTH, which is then stored in the corpora cardiaca–corpora allata complex (CC–CA), the neurohemal organ for PTTH. Release of the neuropeptide takes place at particular developmental stages and is under control of a series of physiological factors, such as the nutritional state of the animal but is also influenced by certain environmental cues, such as photoperiod and time of day. After release, PTTH activates the PGs of larval and pupal animals to synthesize and secrete E, which is finally converted to the biologically active 20E in tissues peripheral to the PGs (as reviewed by Gilbert et al. [40]; Gilbert et al. [39]; Rybczynski [97]; Huang et al. [54]). In the last few decades, a lot of research has been done on the PTTH signal transductory cascade in the PGs of *Bombyx* and *Manduca*. In this review we will provide a short summary thereof. After its secretion into the hemolymph, PTTH binds to a receptor located at the cell membrane surface of the PGs (Fig. 1). Although this receptor is still unknown, recent reports speculate on its identity. During a screening targeting this receptor, Nagata et al. [87] reported on a new receptor cDNA in the PGs of *B. mori*. Although confirmation still requires further analysis, their first data suggest it to be a strong candidate for the PTTH receptor belonging to the family of G-protein coupled receptors (GPCRs). Although the PTTH receptor remains as yet uncharacterized, several downstream signaling pathways activated by PTTH have been identified. Binding of PTTH to its receptor at the cell membrane surface causes an influx of extracellular Ca^{2+}. As shown in Fig. 1, this in turn results in a rapid increase in cAMP content with the help of a Ca^{2+}-calmodulin dependent adenylate cyclase