I.9 Neurohormonal Regulation of Molting and Metamorphosis in the Tobacco Hornworm, *Manduca sexta*

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1. Introduction

The endocrine regulation of insect postembryonic development is generally thought to involve three hormones: the prothoracicotropic hormone (PTTH), a cerebral neuropeptide; the steroid hormone 20-hydroxyecdysone; and juvenile hormone, an aliphatic sequiterpene [1]. PTTH is produced by one neurosecretory cell in each hemisphere of the insect brain [2], while 20-hydroxyecdysone is derived from the ecdysone produced by the prothoracic glands (PG) in response to PTTH [3, 4]. Juvenile hormone exists in four homologous forms (JH 0, I, II, and III) and is produced by corpora allata (CA) [5]. Although the integration of release of the hormones is critical for their roles in the regulation of insect molting and metamorphosis, the pivot of this regulatory mechanism is the periodic release of PTTH in response to environmental cues. Ecdysone is synthesized and released by the PG in response to PTTH and is then hydroxylated by peripheral tissues to 20-hydroxyecdysone, the hormone that elicits molting. JH acts in conjunction with 20-hydroxyecdysone, presumably at the level of the target tissues to determine the character of the molt [1].

In contrast to what is known of the neuroendocrine regulation of ecdysone synthesis by the PG, there is a lack of quantitative information available regarding the regulatory mechanisms involved in the control of CA activity [6]. Just recently however, evidence has been obtained that supports the concept of neuroendocrine regulation of the CA during larval-pupal development (Sect. 3). The complexity of this regulatory system may prove to be greater than that of the PTTH-PG axis, since both stimulatory neurohormones (allatotropins) and inhibitory neurohormones (allatohibins) have been implicated in the control of the CA. The demonstration that cerebral neurohormones control CA function would make the brain the control center for the regulation of molting and metamorphosis. In response to specific cues, either extrinsic or intrinsic, the brain would regulate the synthesis, transport, and release of the neurohormones acting on the PG and CA, and thus control the progression of postembryonic development. There is already a considerable body of information concerning the control of PTTH release by extrinsic and intrinsic cues [7], and it is likely that similar, if not identical, cues could control the release of neurohormones regulating the CA [6].

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In addition to primary regulation by cerebral neurohormones, there is compelling evidence that other, so-called secondary effectors, such as temperature, photoperiod, and hormones also modulate the activity of the PG and CA [8]. Regulation of the glands is, therefore, the end result of the interactions of both primary and secondary effectors that act in a temporal, quantitative, and qualitative manner. Although the secondary regulators are clearly important, neurohormones remain the primary elements in control of glandular activity in insects, as they are in vertebrate and other comparable invertebrate systems. It is because of their predominant contribution to the regulation of insect postembryonic development that we are interested in elucidating their physiological function and chemical nature. This contribution summarizes some of our research on those neurohormones in the tobacco hornworm, *Manduca sexta*, which act as primary effectors of molting and metamorphosis, and this will cover: (1) purification of the prothoracicotropins (PTTH); (2) studies of the mechanism of PTTH action; (3) development of an experimental paradigm for probing putative neuroendocrine regulation of the CA; and (4) evidence arguing for the existence of a cerebral allatotropin and allatothibin.

2. The Prothoracicotropins

2.1 In Vitro Assay for PTTH

In the field of endocrinology, progress has often been dictated by available assays for detecting and quantifying a particular hormone. This has clearly been the case with insect neurohormones, and thus, for our investigations of the neuroendocrine regulation of insect postembryonic development, the aim was to devise assays that would facilitate the quantitative assessment of neurohormone activity. For reasons previously elaborated we chose an in vitro approach and developed an in vitro assay for PTTH [4, 9, 10]. Since this assay is specific, rapid, and yields reproducible and accurate results, it has proven invaluable in our pursuit of an understanding of the chemistry and endocrine physiology of PTTH. Larvae and pupae of the sphingid moth *Manduca sexta* were used to develop the PTTH assay because so much is known about the developmental endocrinology of this species compared to other insects and because certain structural features of the brain-PG axis facilitated the development of the in vitro assay. Basically, the assay consists of the incubation of one PG of a pair with PTTH (the experimental gland) and the contralateral gland without PTTH (the control gland). Activation of the PG by PTTH is then detected as an increased rate of ecdysone synthesis by the experimental PG. This paired gland approach is possible because rates of ecdysone synthesis by the members of a gland pair from the same animal are equivalent. Activation of a PG by PTTH is expressed as an activation ratio (Ar), which represents the quantity of ecdysone synthesized by the experimental gland divided by the amount synthesized by the control gland during the incubation period. This approach was implemented to minimize the inherent variation in the activities of the PG of different animals, since this variation dampened the detectable activation response of the experimental glands. Since originally developed, the PTTH assay has been modified [10] to enable quantifica-