Insect physiology: **The emerging story of ecdysis**

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The discovery of a new insect peptide hormone that triggers ecdysis – shedding of an old cuticle – has revealed hidden layers of intricacy about an insect behavior previously thought to be mediated by a single neuropeptide.

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In mammals, regulatory interactions between hormones are the basis of complex networks of negative and positive feedback loops, which ultimately culminate in diverse physiological and behavioral effects. Invertebrate behaviors, such as egg-laying in *Aplysia*, have also been shown to require complex hormonal mediation [1]. Perhaps, then, it is not surprising that recent experiments on an insect behavior that was once thought to be initiated by a single peptide have uncovered previously unappreciated interactions and regulatory mechanisms.

All insects have an inflexible exoskeleton, or cuticle, which they need periodically to replace in order to grow and develop. The final step of this molting process is a series of stereotyped movements which result in the shedding of the old cuticle. These movements can be divided into two discrete behaviors, known as pre-ecdysis and ecdysis [2]. Both of these behaviors have been shown to be triggered by a neuropeptide known as eclosion hormone (EH). Although first discovered through its ability to trigger adult ecdysis, which has been given the specialized term 'eclosion', EH was subsequently shown to trigger ecdysis at the larval, pupal and adult molts [3].

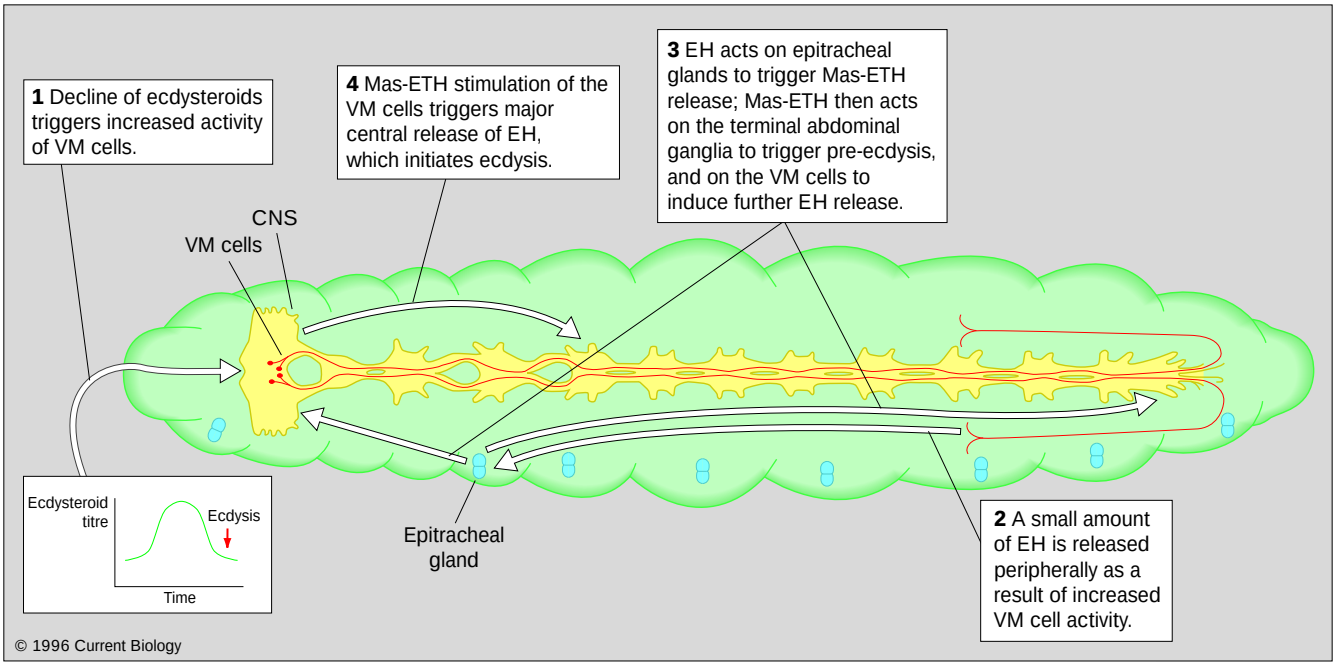
EH is found in two pairs of cells in the insect brain, the VM cells, which project posteriorly along the ventral nervous system to both central and peripheral release sites. For over twenty-five years since its original characterization, EH has been assigned sole responsibility for initiating and coordinating the events involved in ecdysis, and has been assumed to act directly on the central nervous system (CNS) to trigger the behavior [3]. Earlier this year, the discovery of a new peptide was reported that is also capable of triggering pre-ecdysis and ecdysis behaviors [4]. This peptide has been named *Manduca sexta* ecdysis-triggering hormone (Mas-ETH). We shall describe new observations pertaining to the role of EH and Mas-ETH in these behaviors, and present a model which could explain how these two hormones interact.

In their recent paper, Žitňan *et al.* [4] reported the isolation of Mas-ETH from epitracheal glands of the moth *M. sexta*, which are located on tracheae near the spiracles of each segment. Injections of Mas-ETH induced pre-ecdysis and ecdysis, with a shorter latency between the time of injection and initiation of the behavior than there is with EH injections. In addition, Mas-ETH, unlike EH, was shown to induce these motor patterns in isolated central nervous systems *in vitro*. Perhaps most intriguing of all is the preliminary observation that extracts containing EH can cause both pre-ecdysis and ecdysis motor patterns in isolated central nervous systems if freshly dissected epitracheal glands are added to the bathing medium [4]. This observation led the authors to suggest that EH does not act directly on the CNS to trigger pre-ecdysis and ecdysis, as was thought previously, but rather acts as a Mas-ETH-releasing hormone. This model predicts that Mas-ETH acts directly on the CNS to trigger both behaviors.

Nevertheless, some existing published data and some very recent unpublished data suggest that the relationship between EH and Mas-ETH is more complicated than the interpretation presented above. The hypothesis presented by Žitňan *et al.* [4] predicts a requirement for blood-borne EH to elicit Mas-ETH release from the epitracheal glands. Hewes and Truman [5], however, have shown that, when the peripheral EH-release sites are removed, ecdysis behavior occurs at the expected time. Thus it appears that centrally released EH is sufficient to trigger ecdysis and that blood-borne EH is not necessary for initiating ecdysis. Novicki and Weeks (personal communication) have additionally shown that, when the brain is severed from the rest of the nervous system, larvae undergo pre-ecdysis but not ecdysis at the expected time, and there is no measurable EH or Mas-ETH bioactivity in the blood. Furthermore, using a decrease in EH immunoreactivity as a measure of EH release, Truman and Ewer (personal communication) have shown that pre-ecdysis behavior precedes the major release of EH by 25–30 minutes. These results are confusing if blood-borne EH is necessary to stimulate the release of Mas-ETH to induce both pre-ecdysis and ecdysis motor patterns. It is thus unclear how pre-ecdysis behavior is initiated *in vivo*.

Experiments on de-brained larvae not only demonstrate that blood-borne EH is unnecessary for the initiation of pre-ecdysis and ecdysis behavior, but also highlight the role of the brain. Novicki and Weeks (personal communication) have found that, when de-brained larvae are injected with EH, they undergo precocious pre-ecdysis behavior and never show ecdysis. The absence of ecdysis

Figure 1



Proposed model for the interactions of EH and Mas-ETH and their roles in triggering pre-ecdysis and ecdysis behaviors (see text for details).

motor patterns in the debrained animals suggests the requirement for a central pathway in the initiation of ecdysis behavior only. Interestingly, this separation of ecdysis and pre-ecdysis behaviors is also apparent in the sensitivity of the isolated nervous system to Mas-ETH, as a ten-fold higher concentration of Mas-ETH is required to trigger ecdysis than is required to trigger pre-ecdysis [4].

In the process of further evaluating the relationship between EH and Mas-ETH, yet another layer of complexity has been revealed. Žitňan *et al.* [4] suggest that EH causes the release of Mas-ETH. Preliminary results from James Truman's laboratory indicate that injected EH causes a major release of endogenous EH from both peripheral and central sites, as measured by a decline in EH immunoreactivity. Perhaps even more surprisingly, injections of extracts containing Mas-ETH were also found to cause a major release of EH from the VM cells. Also, Truman and Ewer (personal communication) have found that, at the same time that the VM cells release their stores of EH, there is an increase in cyclic (c)GMP immunoreactivity in the epitracheal glands in conjunction with a disappearance of peptide immunoreactivity from these cells. The increase in cGMP is an important observation, as previous studies have shown that cGMP mediates the response to EH [6]. Together, these findings seem to demonstrate the induction of a positive feedback cycle, with each peptide causing the release of the other. Such a cycle is reminiscent of the process of mammalian ovulation in which estradiol and pituitary hormones also form a positive feedback loop.

The question remains how all of these disparate findings fit together to explain the sequence of events that triggers pre-ecdysis and ecdysis behaviors in insects. We propose the following model that is consistent with the available data and is shown in Figure 1. The decline in the steroid hormones, the ecdysteroids, which occurs at the end of a molt has been shown to be necessary for the initiation of EH release, pre-ecdysis and ecdysis [3]. This decline in ecdysteroid level gradually increases the excitability and firing rate of the VM cells [7]. It is possible that the activation of the VM cells results in a small amount of EH being released into the circulation shortly before the onset of pre-ecdysis behavior, and thus triggers the release of a small amount of Mas-ETH. Mas-ETH then could be responsible both for initiating pre-ecdysis behavior by acting directly on the nervous system, and for initiating ecdysis behavior indirectly by causing a more substantial release of EH from the VM cells.

In the normal intact animal, the release of these initial levels of Mas-ETH, perhaps in combination with other peptides, could result in a positive feedback cascade that would culminate in the release of almost all of the stores of EH and Mas-ETH at about 20–30 minutes into pre-ecdysis. In this scenario, Mas-ETH is responsible for initiating pre-ecdysis behavior and causing the central release of

EH, whereas EH released centrally from the VM cells has ultimate responsibility for triggering ecdysis behavior. This model, although it explains the current observations, does not address the problem of sensitivity to Mas-ETH, as the amount of Mas-ETH required to produce pre-ecdysis motor patterns *in vitro* is actually quite high [4]. As it seems that most of the Mas-ETH is released after pre-ecdysis has begun, one might speculate, as suggested by Žitňan *et al.* [4], that other bioactive fractions from the epitracheal glands work in conjunction with Mas-ETH to initiate pre-ecdysis behavior and to cause central EH release.

In addition to triggering ecdysis and Mas-ETH release, EH has been shown to have other effects on the CNS at the end of the molt. For instance, EH causes an increase in cGMP just prior to ecdysis in a population of 50 identified neurons in the ventral ganglia [8]. Also, recent evidence from our laboratory demonstrates that, in response to EH, there is an increase in cGMP immunoreactivity in a neurohemal site of isolated pupal abdominal nervous systems [9]. One possible role for the increase in cGMP in this neurohemal site is to modulate the release of additional peptides in a similar manner to the proposed role of EH as a Mas-ETH-releasing hormone. Thus, the perceived role of EH in ecdysis may grow as it emerges as a coordinator of the diverse physiological events surrounding ecdysis behavior [3].

Although the role of EH in ecdysis behavior is much more complicated than was thought originally, the recent discovery of Mas-ETH and its relationship to EH promises to provide fertile ground for exploring regulatory peptide systems in insects. We have presented a model which takes the new observations into account and delineates a testable hypothesis about the inter-relationship of EH and Mas-ETH. We have also shown that EH may modulate other events at the time of ecdysis and thus serve to regulate physiological changes occurring at this time. The picture that has emerged from the recent flurry of activity is that pre-ecdysis and ecdysis behaviors have different requirements for descending signals from the brain, that EH and Mas-ETH seem to be part of a positive feedback loop, and that the role of EH may include coordination of the release of other peptides during ecdysis. Clearly, there are many layers of the story of peptide-mediated ecdysis behavior that remain to be shed.

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