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## Peptide and Steroid Regulation of Muscle Degeneration in an Insect

Abstract. *Two types of cell death occur in the intersegmental muscles of the giant silkmoth* Antheraea polyphemus. *The first results from a slow atrophy of the fibers, and the second is a rapid, programmed dissolution of the muscle. Both types appear to be mediated by endocrine factors. The slow atrophy is brought about by the decline in the steroid molting hormone 20-hydroxyecdysone and can be prevented with exogenous steroid. The rapid degeneration is triggered by the peptide eclosion hormone, but the sensitivity of the muscle to the peptide depends on the history of exposure of the muscle to 20-hydroxyecdysone.*

Cell death can serve as a required step in the normal differentiation of tissues (*1*) or as the final event in a pathological condition (*2*). The terminal events in cell death have been examined in detail, but few studies have elucidated the factors responsible for its initiation. We now describe a system in which a set of muscles remains healthy, goes through a wasting atrophy, or shows a programmed degeneration, depending on endocrine treatment.

The intersegmental muscles (ISM) of the giant silkmoth *Antheraea polyphemus* have served as a model system for the exploration of events associated with cell death (*3*). These muscles span the fourth through sixth abdominal segments and are essentially the only skeletal muscles in the diapausing pupa. They assist the adult in the emergence (eclosion) from the pupal cuticle, and then they undergo rapid degeneration (*4*).

The degeneration of the ISM is associated with some event that occurs around the time of adult eclosion. Lockshin (*5*) showed that when abdomens were isolated from *A. polyphemus* just before emergence of the adults, the muscles were routinely preserved. By contrast, isolation at or after eclosion resulted in normal breakdown of the ISM. He concluded that a signal from the anterior end of the animal around the time of eclosion was the initiating factor in muscle death. Using muscle weight as an index (*6*), we compared the fate of the ISM in control animals and isolated abdomens (Fig. 1A). The loss of muscle weight in control animals was precipitous; contractility was lost by 20 hours (*7*), and the muscles were reduced to flimsy bags of membranes by 25 hours. In the isolated abdomens, the muscles were preserved well past their normal time of death. The ISM from the isolated preparations underwent a gradual atrophy, as indicated by a loss in dry weight and a reduction in the diameter of individual muscle fibers, but they remained contractile until about 6 days after isolation. This slow atrophy appears to be a continuation of an exponential decline in muscle mass that normally begins 2 days before eclosion (*8*).

Adult eclosion in the silkmoths is triggered by a peptide hormone (*9*) released from centers in the head shortly before eclosion. Isolation of the abdomen before this time would prevent contact of the ISM with this peptide. Injection of eclosion hormone (*10*) into abdomens isolated on the day of eclosion, but before the release of the endogenous hormone, resulted in the rapid breakdown of the muscles (Fig. 1A). This degeneration was qualitatively and quantitatively

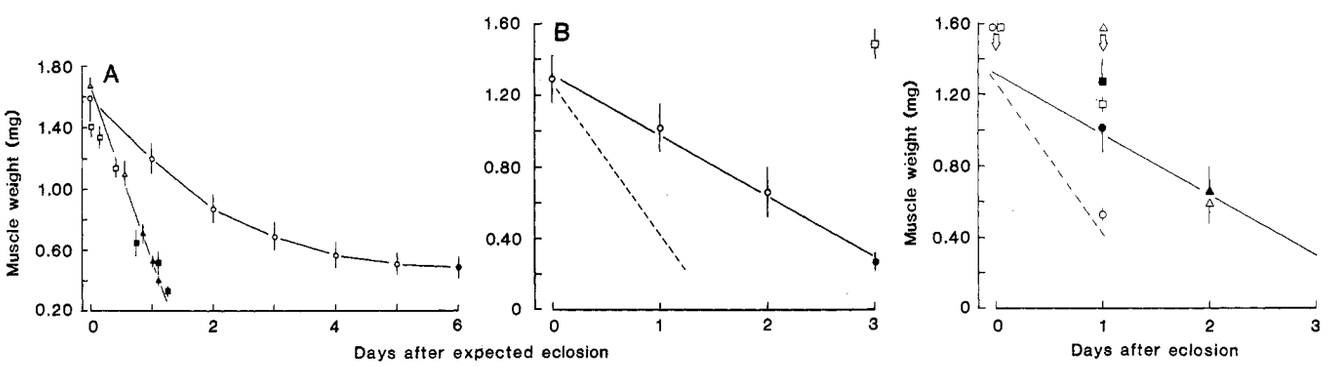


Fig. 1 (left). Effects of endocrine treatments on the degeneration of the intersegmental muscles as quantified by the loss of dry weight. Open symbols represent contractile muscles and closed symbols, noncontractile muscles. (A) Role of eclosion hormone. (□, ■) Intact, naturally emerging adults; (○, ●) abdomens isolated from animals on the day of emergence, but before release of eclosion hormone; and (△, ▲) abdomens injected with 1 unit of eclosion hormone after isolation. A least-squares regression line is drawn for isolated abdomens injected with eclosion hormone. (B) Role of ecdysteroids. (○, ●) Animals receiving an injection of 25 μg of 20-hydroxyecdysone on the day preceding eclosion (day 16). (□) Animals continuously infused with 20-hydroxyecdysone (30 ng/g-hour) beginning 2 days before eclosion (day 15) and continuing until 3 days after expected emergence. The dashed line represents the rate of ISM degeneration found for control animals in (A). Values are means ± S.E. for four or five animals. Fig. 2 (right). Treatment with 20-hydroxyecdysone renders the ISM insensitive to eclosion hormone. The dashed line shows the time course of muscle loss from intact animals or isolated abdomens injected with eclosion hormone (data from Fig. 1A); the solid line shows the weight loss for animals injected with 25 μg of 20-hydroxyecdysone on the day preceding eclosion (data from Fig. 1B). Closed symbols indicate animals injected with Ringer solution; open symbols indicate animals treated with 1 unit of eclosion hormone. (○, ●) Abdomens isolated before eclosion. (□, ■, △, ▲) Intact insects treated with 25 μg of 20-hydroxyecdysone on the day before eclosion (all insects eclosed on schedule). Arrows with associated symbols indicate the time at which various groups were injected with either eclosion hormone or Ringer solution. Values are means ± S.E. for four or five animals.

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identical to that in intact animals. Peptide-induced degeneration appeared to be all-or-none and occurred with a threshold of about 0.1 unit of eclosion hormone. Sensitivity of the muscles to eclosion hormone appears on the last day (day 17) (*11*) of adult development. The ISM of abdomens isolated on the day preceding eclosion (day 16) showed only the slow atrophy typical of isolated abdomens, even when ten times the threshold dose of eclosion hormone was injected.

20-Hydroxyecdysone prevents many of the terminal events of adult development in insects (*12*), including release of eclosion hormone (*13*). Dissection of animals given this steroid revealed little or no degeneration of the ISM. When developing adult moths were given infusions of 20-hydroxyecdysone (30 ng/g-hour) (*14*) starting 2 days before eclosion (day 15), none of the animals emerged, and the ISM, when examined 5 days later, had retained their previous weights (Fig. 1B). A single injection of 20-hydroxyecdysone (25 μg per animal) into normal animals on the day preceding eclosion (day 16) permitted eclosion at the normal time on the following day, but the ISM continued to atrophy slowly rather than to undergo the expected rapid dissolution. Since 20-hydroxyecdysone can prevent the release of eclosion hormone, animals that had been given 20-hydroxyecdysone on the day before eclosion were given a supramaximal dose of eclosion hormone (1 unit), either on the day of expected emergence or 1 day thereafter. In both instances, the ISM failed to show rapid degeneration (Fig. 2). Thus the decline in 20-hydroxyecdysone appears to be essential for the development of sensitivity to eclosion hormone by the muscles.

The finding that the slow atrophy of the muscles was prevented when 20-hydroxyecdysone was injected 2 days before eclosion (day 15), but not when it was injected 1 day before eclosion, also suggested that the slow atrophy may be initiated by a decline in the endogenous ecdysteroid level below some threshold. The ecdysteroid levels in the hemolymph (*15*) declined from a mean ± standard error (S.E.) of 356 ± 61 ng/ml 3 days before eclosion to 86 ± 8 ng/ml 1 day before eclosion. A value of 20-hydroxyecdysone between these two values may represent the threshold necessary to initiate atrophy.

These data suggest a complex endocrine regulation of the fate of the ISM in *A. polyphemus*; the muscles either display a wasting atrophy, with fibers becoming very thin and eventually losing contractility, or a programmed breakdown occurs, with rapid degeneration of muscle. Late in adult development the ISM are maintained by an elevated titer of 20-hydroxyecdysone, which acts as a trophic factor for the muscles; a decline in this steroid appears to cause the muscles to atrophy. Similar steroid-dependent muscle maintenance in the levator ani muscle of rats requires the presence of testosterone for normal function (*16*).

The normal decline in 20-hydroxyecdysone also serves to make the ISM competent to respond to eclosion hormone. When 20-hydroxyecdysone titers are high, either earlier in adult development or as a result of exogenous hormone injection, the muscles do not degenerate in response to eclosion hormone. Thus, steroid withdrawal is necessary for peptide action in this system. The mechanism of this regulation is not known, but may be similar to that in rat myometrium, where a sharp decline in the levels of circulating progesterone before parturition induces the production of oxytocin receptors (*17*). After the decline of ecdysteroids in the silkmoth, eclosion hormone triggers the programmed cell death of the ISM, which is characterized by an increase in the number of muscle lysosomes, loss of myofibrillar proteins, and involution of the fibers (*3*). Thus, a peptide and a steroid interact with the ISM to regulate the mode of cell death.

LAWRENCE M. SCHWARTZ
JAMES W. TRUMAN

*Department of Zoology,*
*University of Washington,*
*Seattle 98195*

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6. Diapausing pupa of *Antheraea polyphemus* were purchased from suppliers and stored at 4°C. As needed, animals were transferred to an incubator at 25°C and kept at a photoperiodic cycle of 17 hours of light and 7 hours of darkness. For some experiments, abdomens were isolated from the thorax with a hemostat before the release of eclosion hormone on the last day of adult development. Only males were used in this study. At the time of assay the lateral ISM were removed from animals, placed on foil of known weight, dried at 60°C and weighed on a Mettler M5 balance.
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10. The eclosion hormone used was either a supernatant of an aqueous extract of pharate (day 18) adult *Manduca sexta* corpora cardiaca, which had been heated at 80°C for 5 minutes, or a more highly purified preparation of this extract, which had been subjected to gel filtration and narrow-range isoelectric focusing as in P. H. Taghert, J. W. Truman, and S. E. Reynolds [*J. Exp. Biol.* **88**, 339 (1980)]. A unit of eclosion hormone is defined as the amount of hormonal activity found in one pair of pharate adult *Manduca sexta* corpora cardiaca.
11. Animals were staged according to distinct morphological markers [D. R. Walters, *J. Exp. Zool.* **174**, 441 (1970)]. Two days before emergence (day 15), animals have an ochre face and two distinct orange bands across the wings. On day 16, most of the pupal endocuticle has been degraded and the wings are very dark. On day 17, developing adults have a thin, crispy exocuticle and have resorbed their molting fluid.
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15. Ecdysteroid titers were determined by radioimmunoassay according to the procedure of E. S. Chang and J. D. O'Connor, in *Methods of Hormone Radioimmunoassay*, B. M. Jaffe and H. R. Behrman, Eds. (Academic Press, New York, 1979), p. 797. Data are expressed as 20-hydroxyecdysone equivalents. The antibody was provided by J. D. O'Connor.
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## Jumping Chickens: Relevance to Hazard in Humans

Sanderson and Rogers have reported the results of a study on the behavioral effects in chickens given "environmentally relevant doses" of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and they postulated that humans would be three times more sensitive to this herbicide than chicks (*1*). The conclusions drawn from this study do not reflect a valid evaluation of the data because (i) the doses were not environmentally relevant for bird eggs, much less for humans, and (ii) chick embryo data are not a suitable model for safety assessment in humans and are not used by any government agencies for this purpose.

Recent studies by Lavy *et al.* (*2*) have shown that the maximum exposure to


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