

Crustacean Hyperglycemic Hormone, a possible endocrine regulator of ecdysis in the tobacco hornworm, *Manduca sexta*

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ABSTRACT

Ecdysis, the precisely timed removal of the old cuticle, occurs in a wide range of invertebrates and is both neurally and hormonally regulated. Understanding the regulation of ecdysis could lend pertinent details to the fundamental workings of the endocrine system and neural modulation of behavior. Crustacean Hyperglycemic Hormone was initially identified in crabs for its ability to increase blood glucose levels and has recently been implicated in the regulation of ecdysis in crabs as well as having roles in water and pH regulation. The family of Crustacean Hyperglycemic Hormones (CHHs) has been localized to the brain, major endocrine cells, and transverse nerves of crustaceans. We have identified CHH-like immunoreactivity in the moth, *Manduca sexta*, to sera specific for 2 different CHH peptides isolated from two crab species, *Carcinus maenas* and *Cancer pagurus*. This staining is at least partially blocked by both peptides, suggesting that the peptides in *Manduca* may be related peptides from a CHH peptide family. Staining was visualized in the neurosecretory cells of the brain and in the endocrine glands that receive input from this region. It was also noted in transverse nerves of abdominal ganglia, which provide a second source of hormone to the blood. CHH's role in ecdysis and water balance are currently under investigation.

INTRODUCTION

Neuroendocrine regulation of physiological systems is implicated in diverse processes ranging from control of digestion to reproduction and behavior. Mechanisms, including the hormones and neurotransmitters involved, appear to be conserved in vertebrates as well as invertebrates (Siviter *et al.* 2000). Our lab's focus is to identify novel hormones that are involved in ecdysis, the programmed shedding of the old cuticle. Ecdysis behavior is absolutely necessary for continued growth and development, and involves neural control from central and peripheral sources as well as hormonal control from endocrine centers. Our invertebrate model, *Manduca sexta*, is ideal for studying ecdysis because (i) its endocrine system is well characterized, (ii) it has identifiable cells from preparation to preparation, and (iii) it is very amenable to surgical manipulation.

The invertebrate regulatory peptide, Crustacean Hyperglycemic Hormone (CHH), has been implicated in control of their ecdysis. CHH levels were shown in previous studies to rise sharply in concert with the onset of ecdysis in the crab. CHH is a large peptide hormone - 72 amino acids in length - and is named for its initial identification and characterization in the crab, *Carcinus maenas*, based on its hyperglycemic effects in the blood (Kegel *et al.* 1983). CHH has since been identified in many crustaceans (Dirksen *et al.* 2001), and related peptides have been found in insects (Macins *et al.* 99), (Endo 99). In addition to regulation of carbohydrate metabolism, CHH is involved with electrolyte balance (Chung *et al.* 1998), and now possibly insect ecdysis.

This preliminary study will characterize the distribution and specificity of CHH antibody staining in larval *M. sexta*. The ultimate goal is to implicate CHH in insect ecdysis and to elucidate the function of CHH in the context of the ecdysis motor program.

Materials and Methods

Immunohistochemistry
Primary antibody: Rabbit anti-*Cancer* CHH and Rabbit anti-*Carcinus* CHH (1:1000)
Secondary Antibody: peroxidase-conjugated Donkey anti-Rabbit IgG (1:500)
Color reaction: Diaminobenzidine (DAB) Tetrahydrochloride
Blocking:
Preabsorption: *Cancer maenas* 0.2pmol and *Carcinus pagurus* 0.3pmol peptide

C. Pagurus CHH like immunoreactivity in *Manduca sexta* brain and Corpora Cardiacia

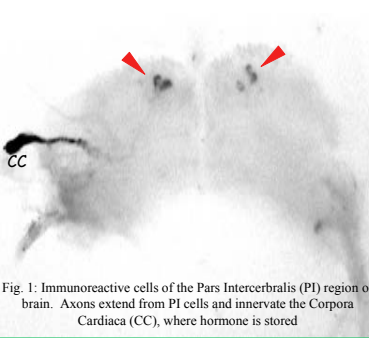


Fig. 1: Immunoreactive cells of the Pars Intercommissuralis (PI) region of brain. Axons extend from PI cells and innervate the Corpora Cardiacia (CC), where hormone is stored

C. maenas CHH like immunoreactivity in *Manduca sexta* brain and Corpora Cardiacia

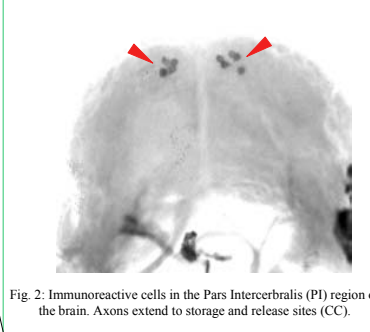


Fig. 2: Immunoreactive cells in the Pars Intercommissuralis (PI) region of the brain. Axons extend to storage and release sites (CC).

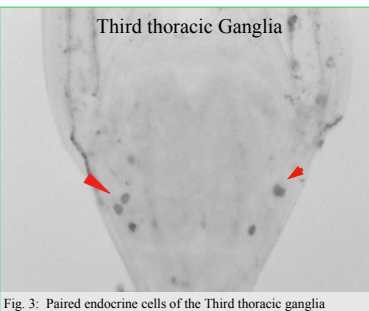


Fig. 3: Paired endocrine cells of the Third thoracic ganglia

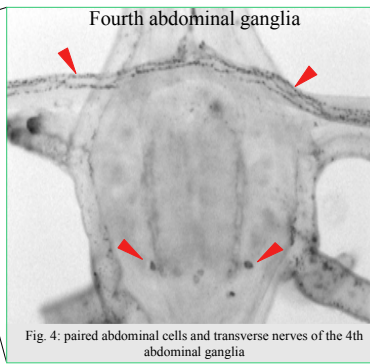


Fig. 4: paired abdominal cells and transverse nerves of the 4th abdominal ganglia

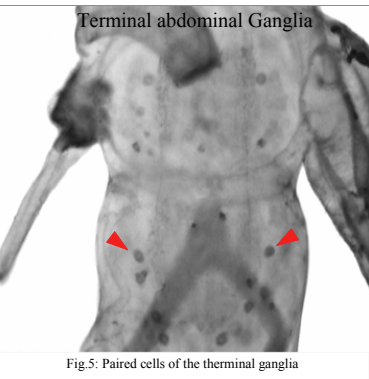


Fig.5: Paired cells of the terminal ganglia

Binding Specificity

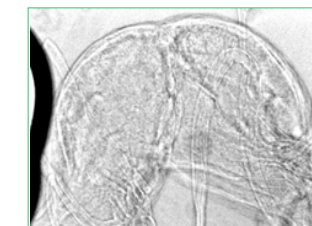


Fig. 6: Pre-incubation with *C. pagurus* CHH fully blocks staining with *C. pagurus* CHH antibody in *Manduca sexta* brain.

DISCUSSION

1. We have determined that antibodies for CHH using *Carcinus Maenas* and *Cancer Pagurus* sequences are immunoreactive in *M. Sexta*.
2. There are likely other roles for CHH in *M. sexta*, since CHH-like immunoreactivity is also localized to the CC (insect endocrine gland), endocrine cells of abdominal ganglia as well as transverse nerves. CHH has also been localized to the PI region of the CNS.
3. Initial blocking experiments indicate that the two CHH peptides partially block each other's antisera.

FUTURE DIRECTIONS

1. Definitely determine CHH specificity.
2. Complete a developmental profile of CHH distribution; including stages just prior to, during and after ecdysis.
3. Perform physiological bioassays to verify or describe novel functions for CHH in the insect.
 - i. Radioimmunoassay for CHH in hemolymph
 - ii. Ion and water transport assays in gut or Malpighian tubules

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