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

Christina C. Harris, Marilyn Asuncion-Uchi, Megumi Fuse

Department of Biology, San Francisco State University, San Francisco, CA 94132

**ABSTRACT**

Ecdysis, the precisely timed removal of the old cuticle, is a ubiquitous process occurring across a wide range of invertebrates and has been studied extensively in both model and non-model systems. The regulation of ecdysis is fundamental to the survival of all insects, and recent studies have begun to elucidate the complex interplay of endocrine and neural mechanisms involved. Crustacean Hyperglycemic Hormone (CHH) is a key regulatory peptide in crustaceans, first identified in the shore crab *Carcinus maenas* based on its hyperglycemic effects in the blood (Kegel et al., 1983). CHH has since been identified in many crustaceans (Dircksen et al., 1999), (Endo 1999). In addition to regulation of carbohydrate metabolism, CHH is involved with electrolyte balance (Chung et al., 2001), and related peptides have been found in invertebrates (Macinnes et al, 1999), (Endo 99). In addition to regulation of carbohydrate metabolism, CHH is involved with electrolyte balance (Chung et al., 1998), and possibly insect ecdysis.

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 known for its initial characterization in the crab, *Carcinus Mauritanicus*, based on its hyperglycemic effects in the blood (Kegel et al., 1983). CHH has since been identified in many crustaceans (Dircksen et al., 1999), and related peptides have been found in invertebrates (Macinnes et al., 1999), (Endo 99). In addition to regulation of carbohydrate metabolism, CHH is involved with electrolyte balance (Chung et al., 1998), and possibly insect ecdysis.

This preliminary study will characterize the distribution and specificity of CHH immunoreactivity 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 ecdysial ecdysis program.

**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 (Srivast, 2009). 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 known for its initial characterization in the crab, *Carcinus Mauritanicus*, based on its hyperglycemic effects in the blood (Kegel et al., 1983). CHH has since been identified in many crustaceans (Dircksen et al., 1999), and related peptides have been found in invertebrates (Macinnes et al., 1999), (Endo 99). In addition to regulation of carbohydrate metabolism, CHH is involved with electrolyte balance (Chung et al., 1998), and possibly insect ecdysis.

This preliminary study will characterize the distribution and specificity of CHH immunoreactivity 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 ecdysial ecdysis program.

**MATERIALS AND METHODS**

**Immunohistochemistry**

Primary antibody: Rabbit anti-Carcinus 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 moose CHH peptide and Carcass Pagurus CHH peptide

**ACKNOWLEDGEMENTS**

This research was funded in part through (1) USDA research grant #693973 (M.F.), 2) “Research Infrastructure in Minority Institutions” award from the National Center for Minority Health and Health Disparities. P20 MD00262 (M.F.), 3) start up funds from SFSU (M.F.), and 4) Student Enrichment Opportunities (CEO, MBA, BERO grant #R25GM64078-02 (CCH)).

**REFERENCES**


