

# In Vitro Activity of the Ecdysis Triggering Hormone (ETH): Establishing a Structure Activity Relationship

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Table 2: *In vitro* profile for strong cGMPPr in CNS

Analog	SEG stah	TG Stah	Abdominal stah
28mer	9/9 1/9	9/9	8/9 1/9
12mer	6/10 3/10	9/11 1/11	6/11 3/11
7mer	3/3	3/3	1/3 2/3
6mer	0/4 2/4	0/4 2/4	0/4 0/4

Table 3: *In vitro* electrophysiological profile and characterization of response for various analogs

Analog	Ecdysis response	Characteristics
28mer	5/7	Normal timing for Pre-Ecdysis / Ecdysis
12mer	5/5	Normal onset of ecdysis; Shorter transition but a sustained robust ecdysis pattern
7mer	3/4	Normal onset of ecdysis; Longer transition period and a less organized, shorter ecdysis pulse

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## Results

Table 1: Minimal moieties needed for activity at normal and high doses

ETH analog	Size	Biological Activity (Y/N)	Dose (nmol)	Normal timing (Y/N)	# animals
SPFDGGMGIVYKTKNFKNIPRK-NH2	28 mer	Y	0.1	Y	60
SPFDGGMGIVYKTKNFKNIPRK-NH2	28 mer	Y	0.1	Y	19
YVYKTKNFKNIPRK-NH2	12 mer	Y	0.1	Y	22
VYKTKNFKNIPRK-NH2	11 mer	N	0.1	N	14
VKTKNFKNIPRK-NH2	10 mer	Y	100	Y	3/3
IKTKNFKNIPRK-NH2	9 mer	N	0.1	N	6
IKTKNFKNIPRK-NH2	9 mer	Y	100	N	4/2
KNIPRK-NH2	6 mer	N	100	N	3/3
KNIPRK-NH2	6 mer	N	100	N	3/4

Figure 1: cGMPPr after analog incubation by 7 mer analog at physiological concentrations

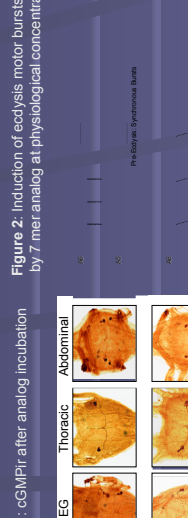


Figure 2: Induction of ecdysis motor bursts by 7 mer analog at physiological concentrations



Summary:

Size	Activity <i>In vivo</i>	cGMPPr ( <i>In vivo</i> )	Electrophysiology (neuron activation)
28 Mer	Y	Y	5/7 (normal)
12 Mer	Y	Y	5/5 (normal)
11 Mer	Y (high)	Y	5/5 (normal)
7 Mer	Y (high)	(Y)	3/4 (abnormal)
6 Mer	N (high)	N	
5 Mer	N (high)	N	

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Future Directions:

- Continue to test all analogs
- Alanine substitutions (eg. PETH vs ETH)
- Correlate differences in *in vitro* stained preparations to burst patterns

## 1

**Introduction**

Neuropeptides play pivotal roles in modulating various physiological processes in animals, including growth, development, digestion, and movement. In these instances, they act on receptors at target sites where they elicit biological activity.

Ecdysis is a motor behavior that occurs in many invertebrates, which accommodates their growth and subsequent progression through various developmental stages. This behavior culminates in the shedding of the cuticle (skin), and is under tight neuroendocrine control.

In insect *Manduca sexta* serves as a simple model in studying the modulation of complex behaviors by neuropeptides due to its ease-of-use experimentally, its well-characterized CNS, and endocrine system, and identifiable neurons. Finally, ecdysis can be readily observed by the stereotyped wave of asynchronous muscle contractions that propagates through the entire length of the animal, as well as *in vitro*, by monitoring larval activation immunohistochemically and electrophysiologically.

Several neuropeptides are involved in orchestrating the ecdysis cascade in *Manduca* including Pre-Ecdysis Triggering Hormone (PETH), which causes loosening of the old cuticle, and Ecdysis Triggering Hormone (ETH), which is necessary for both loosening and shedding of a old cuticle.

Both PETH and ETH share a high degree of structural similarity, but only ETH causes shedding of the animals' old cuticle via a cGMP-mediated mechanism. We are interested in how ETH modulates ecdysis and more specifically, in its structure-activity relationship:



**Hypothesis:** The carboxy-terminal domain of ETH is most important to its biological activity.

**Rationale:**

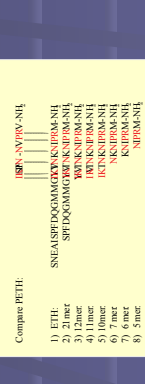
1. ETH is amidated at its carboxy-terminus

2. De-amidated ETH does not cause biological activity (C Wells, unpublished data)

## 2

**Materials and Methods**

### 1. Design Truncations of ETH



### 2. *In vivo* ETH analog injection



### 3. Incubate isolated CNS in ETH analogs and assess ecdysis responses



## 3

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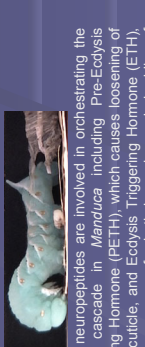
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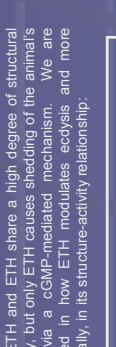
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Goal: To determine the minimal amino acid sequence of ETH necessary for biological activity (ecdysis).