

**Pattern of Neuronal Apoptosis in Silk Moth Terminal Abdominal  
Ganglion and Apoptotic Induction by 20-Hydroxyecdysone *in vivo* and  
*in vitro***

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Apoptosis in the terminal abdominal ganglion (TAG) of the silk moth *Bombyx mori* was studied, focusing on pattern of apoptosis and effects of 20-hydroxyecdysone (20E) *in vivo* and *in vitro*. To demonstrate a pattern of apoptotic neurons in TAG during metamorphic periods, TAG were isolated from larvae and pupae, and then treated by TUNEL assay. During the 1st to 5th instar larval and wandering larva periods, apoptotic neurons were not found in TAG. Day-1 pupa to day-5 pupae showed a gradual increasing slope of apoptotic neurons. To investigate whether 20E and new protein synthesis triggered apoptosis of TAG neurons *in vivo*, 10ul of 1mM 20E and 100ug/ml cycloheximide (CHX) were injected into twenty four hours before pupation(stage W3-noon) and day-1 pupa, and then isolated TAG was treated by TUNEL assay to detect apoptotic neurons. when 20E was injected into day-3 wandering larva and the day-1 pupa, neuronal apoptosis in TAG occurred further exposure to 20E. Co-injection of 20E and CHX blocked neuronal apoptosis triggered by 20E. Caspase 3 inhibitor is sufficient to inhibit neuronal apoptosis in TAG. To clarify whether 20E induced apoptosis of TAG neurons *in vitro*, dissociated TAG neurons were cultured with only 10 ul of 1mM 20E or with both 10 ul of 1 mM 20E and 100 ug/ml CHX. When isolated neurons of TAG were cultured with only 20E, they showed apoptosis in day-5 during culture. When isolated TAG neurons were cultured with culture both 20E and CHX, they showed apoptosis in the day-8 during culture. The experiment suggests that CHX inhibited neuronal apoptosis triggered by 20E.