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Evidences for Programmed Cell Death in Terminal Abdominal Ganglion of the Silk Moth, Triggered by 20-Hydroxyecdysone *in vivo* and *in vitro*

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The programmed cell death (PCD, or apoptosis) in the terminal abdominal ganglion (TAG) of the silk moth, *Bombyx mori*, was studied *in vivo* and *in vitro*, focusing on pattern of apoptosis and effects of 20-hydroxyecdysone (20E). To demonstrate a pattern of apoptotic neurons in the TAG during the metamorphic periods, the TAGs were isolated from the larvae, wandering prepupae pupae and adults, and then treated by TUNEL assay. To investigate whether 20E triggered apoptosis of TAG neurons *in vivo*, 10 ul of 1mM 20-HE was experimentally injected into the day-3 wandering larvae and then the isolated TAG were treated by TUNEL assay to detect apoptotic neurons. To clarify whether 20E induced apoptosis of TAG neurons *in vitro*, the dissociated TAG neurons were cultured with 10 ul of 1mM 20E or 10 ul of 1 mM 20E and 100 ug cycloheximide in culture media and then treated by TUNEL assay. During the 1st to 3rd instar larval periods, apoptotic neurons were not found in the TAG, but the TAG of 4th instar larvae showed a small number of apoptotic neurons. A few apoptotic neurons were found in the TAG during the 5th instar to the late wandering larvae. Early pupae showed a gradual increasing slope of apoptotic neurons. In the day-3 to day-5 pupae the TAG showed a peak of apoptotic neurons, but in the day-6 and day-7 pupae the apoptotic neurons were not shown in the TAG. When the late wandering larva was experimentally injected with 10 ul of 1mM 20E, the apoptotic neurons were increased in the TAG. When the isolated neurons of TAG were cultured with 20E, they showed apoptosis in the day-10 in culture. When the isolated TAG neurons were cultured with both 20E and cycloheximide, they showed apoptosis in the day-17 in culture, suggesting that the cycloheximide inhibited the neuronal apoptosis triggered by 20E.