

Signaling for Neuronal Apoptosis Triggered by Ecdysone in Silk Moth Brain

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Apoptosis, which is necessary for postembryonic development of insect during metamorphosis, was studied in the postembryonic brain of the silk moth *Bombyx mori*. This study was focused on detection of signaling pathway for neuronal apoptosis triggered by 20-hydroxyecdysone (20E), which was well known as an inducing hormone of insect metamorphosis. To demonstrate whether 20E induced neuronal apoptosis in brain, 20E was injected into the instar larva brains of three specific stages. 20E induced neuronal apoptosis by significantly increasing the number of apoptotic neurons in the brain. To clarify whether 20E induced neuronal apoptosis by stimulating synthesis of new proteins in apoptotic neurons, 20E and cycloheximide (CHX) or 20E and actinomycin D were injected into the fourth instar larvae. Both of them made apoptotic neurons to be reduced in number. To verify whether this cell death result in caspases, a caspase inhibitor was injected into the fourth instar larva and to find out the detail pathway of apoptosis triggered by 20E, 20E was injected into the fourth instar larva and then isolated brain was treated by western blotting with anti-procaspase 3 and anti-cleaved caspase 3. 20E stimulated neuronal apoptosis by inducing cleavage of existing precursor of caspase 3 into active caspase 3. To understand upstream apical caspases, inhibitor of caspase 8, one of apical caspases that is suspected of regulation of caspase 3 in the pathway, was injected into the larva brains. These results showed that 20E triggered synthesis of new proteins in apoptotic neuronal cell and these new proteins activated upstream apical caspase 8. In downstream, apical caspases changed procaspase into active caspase 3. Finally, PCD was completed.