E121

Brain-derived Neurotrophic Factor Promotes Neurite Growth and Survival of Antennal Lobe Neurons from the Silk Moth *Bombyx mori In Vitro*

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Brain-derived neurotrophic factor (BDNF) induced a significant neurite extension of ALprojection neurons from the silk moth *Bombyx mori* in culture on laminin/concanavalin A-coated dishes, in comparison with smaller effect of 20-hydroxyecdysone (20-HE). But effect for neurite extension by 5-hydroxytryptamine (5-HT or serotonin) could not befound. A remarkable increase in number of newly grown branches from the neurites of AL projection neurons was also shown in culture with BDNF and 5-HT, but not with 20-HE. BDNF stimulated to outgrow more increased number of branches than 5-HT. In culture of AL projection neurons with BDNF, 20-HE and 5-HT, they showed the highest survival rate in culture with BDNF. Result from western blotting revealed that BDNF, transported from brain neurons to CA, was secreted into hemolymph. Immunostaining of 1- and 2-stage pupal brains with anti-BDNF antibody resulted in presence of four pairs of large median neurosecretory cells and six pairs of small lateral neurosecretory cells of which axons were innervated to the corpora allata (CA). These results suggest that BDNF, secreting from these brain neurons during metamorphosis, may play a role in development of these AL projection neurons.Indexing words: BDNF, neurite growth, survival, antennal lobe neurons, neurosecretory cells, silk moth

E122

Programmed Cell Death in the Developing Brains of the Silk Moth *Bombyx mori* during Metamorphosis

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Programmed cell death (PCD), apoptosis, occurs frequently during animal development. In vertebrates development and patterning of brain and other tissues depend on signals from adjacent tissues, whereas in insects imaginal brain and other tissues and organs are newly formed mainly from imaginal discs together with apoptosis. PCD eliminates most larval tissues in the pupa and pupal tissues before metamorphosis to the adult. It has been demonstrated that PCD in several tissues including the brain are induced by 20-hydroxyecdysone titers in hemolymph. To confirm neuronal apoptosis pattern in the developing brains from early larvae to late pupae of an insect, PCD in the brain from the silk moth *Bombyx mori* during metamorphosis was investigated by the TUNEL assay. As the insect metamorphoses from a larvae to a pupa and finally to an adult, PCD eliminates neurons whose functions have become obsolete. This phenomenon is particularly apparent in the brains of 5th instar larvae and late pupal brains and the results are described in details.

E123

Modeling Ultradian Rhythmicity of Hypothalamic GnRH Neurons

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Pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus is crucial for the neuroendocrine regulation of pituitary function in the hypothalamic-pituitary-gonadal axis. However, the origin and molecular nature of GnRH pulsatility are still unknown. In the present study, we simulated ultradian rhythmicity by *in silico* modeling approach. Based on the recent study in which pulsatile secretion of GnRH is originated from transcriptional oscillation, a negative feedback-based self-oscillation model at the levels of GnRH mRNA and GnRH secretion was formulated. Furthermore, this model was expanded to examine the network effect with 100 synaptically interconnected neurons. This model exhibits a clear ultradian rhythmicity and synchronization effect as compared to the randomly firing network model. This *in silico*approach together with wet experimental data appear to be valuable in elucidating the molecular nature and origin of biorhythmicity in the complex GnRH neuronal apparatus.

E124

Subcellular Localization of Circadian Clock Gene Products by Recombinant Adenoviral Expression System

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The nuclear translocalization and/or phosphorylation of core clock molecules appear to be important in the regulation of appropriate clock machinery. To study the expression and subcellular localization of clock molecules in SCN 2.2 cells (a rat immortalized SCN cell line), recombinant adenoviruses containing nine clock genes were generated by a gateway cloning system followed by an adenovirus overexpression system. Adenoviruses containing EGFP-tagged clock genes were successfully infected into a variety of cell lines including SCN 2.2 cells with high efficiency. Clock molecules were stably expressed with the expected molecular size. Laser scanning confocal microscopic analysis showed that depending on their characteristics, clock molecules were localized in the cytoplasm and/or the nucleus. Upon serum shock, the intracellular localization of two clock molecules, mPER2 and mCRY2 were monitored in SCN 2.2 cells. The nuclear distribution of mPER2-EGFP was gradually gethered to the specific region of the nucleus, while mCRY2 was resided in the nucleus regardless serum shock stimulation. The recombinant adenvirus-mediated gene delivery system appears a valuable tool for molecular and cellular regulation of clock machinery by overcoming low transfection efficiency in the non-dividing neuronal cells. Furthermore, these resources could be useful for a gene transfer and possibly therapeutic applications *in vivo*.