ENTRAINMENT OF THE CIRCADIAN CLOCK GATING PROTHORACICOTROPIC HORMONE SECRETION IN THE ASIAN COMMA BUTTERFLY, POLYGONIA C-AUREUM L.

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Prothoracicotropic hormone secretion inducing larval ecdysis from the 4th to 5th instar preceds the larval ecdysis by 32 hr at 25°C. The PTTH secretion was gated by a circadian clock in Polygonia.

The acrophase-time of PTTH secretion which was obtained by subtracting 32 hr from the time of larval ecdysis came to on an almost parallel line with a line connecting the mid points of 2-hr to 20-hr light periods in 24-hr LD cycles.

Groups of insects reared under 2-hr to 18-hr light photoperiodic regimens at 25°C were transferred to continuous light or to contimuous dark condition at 25°C and the acrophase-time of PTTH secretion was obtained in these groups addition to the other larval groups raised from the egg stage under resonant conditions consisting of an 8-hr light and an various length (2-hr to 64-hr) dark periods.

The results indicated that the circadian clock gaing PTTH secretion may be set twice a day, at dawn and dusk. At dawn the circadian clock may be reset at CT 0:00, and again set at dusk to give the same time (CT 18:00) amid the dark period of 24-hr LD cycles.

ECDYSTEROIDS DURING EARLY EMBRYOGENESIS IN THE SILKWORM, BOMBYX MORI.

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Ovaries in the silkworm are the site of biosynthesis and accumulation of ecdysteroids, and these accumulated ecdysteroids are transported into eggs.

First, ecdysteroid content of diapause eggs and non-diapause eggs was analyzed by both RIA and reverse phase HPLC. Nine free ecdysteroids and their conjugated forms were detected throughout early embryogenesis. In diapause eggs, most free ecdysteroids remained at original levels, but conjugated forms began to increase with the onset of the diapause. In non-diapause eggs, most free ecdysteroids, including 20-hydroxyecdysone, began to increase as embryogenesis proceeded. In contrast, conjugated forms remained at their original levels.

Next, in order to examine the function of free ecdysteroids in the eggs, 20-hydroxyecdysone was injected into 20-hr prospective diapause eggs. Their developmental fate was changed from the diapause type to the non-diapause type. This fact strongly suggests that the elevation of the titer of 20-hydroxyecdysone is needed to advance embryonic development of the silkworm.

HOW DOES PARASITIC WASP INDUCE GROWTH-BLOCKING PEPTIDE IN THE PARASITIZED ARMYWORM LARVAE?

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Last instar larvae of the armyworm parasitized with the parasitoid wasps, Cotesia kariyai, do not initiate metamorphosis and, ultimately, the wasp larvae emerge from the host larvae about 10 days after parasitization. The developmental arrest can be reproduced by injection of parasitoid ovarian calyx fluid containing the symbiotic virus (polydnavirus) which is normally injected by female wasps into the host at oviposition. A peptidergic factor, growth-blocking peptide (GBP), has been purified from the larval hemolymph of the parasitized armyworm. Injection of GBP into unparasitized last instar larvae of the armyworm clearly retards larval growth and, consequently, delays the onset of pupation of the larvae. Recently, it has been demonstrated that GBP exists in plasma of the virus-injected unparasitized last instar larvae and also in plasma of the penultimate instar larvae of the armyworm. Therefore, it is reasonable to propose that infection of polydnavirus activates GBP gene expression, and the production of GBP retards a normal development of last instar armyworm larvae.

PHYSIOLOGICAL SIGNIFICANCE OF 3-DEHYDROECDYSONE(3dhE) FROM CRAYFISH, *Procambarus Clarkii*

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Finding the dominant ecdysteroid 3dhE from *P. clarkii*, upon *in vitro* culture of Y-organs(1), led us to inquire what the physiological function of 3dhE is. After bilateral eyestalk ablation, the production of 3dhE in Y-organs was increased prior to ecdysone(E) and accompanied by an increase of 20-hydroxyecdysone(20E) in hemolymph.The hormonal response of 3dhE, upon injection into *P.Clarkii*, was comparable to those of E and 20E. When a large amount of 3dhE* was injected into crayfish, most of the unchanged material was excreted within 1h and then its metabolite* (3-*epi*-E,3-*epi*-20E,ecdysteroids'conjugates *etc.*) was observed in the excretion. The major radio isotope in the body was found 3h after administration in the epidermal tissues(with carapace), and after 72h in the hepatopancreas. The epidermal isotope was found to be comprised of 3dhE,E,20E,3-*epi*-E,3-*epi*-20E,polar ecdysteroids and conjugates *etc.*. Ecdysteroids* were separated from the conjugates* by enzymatic digestion (*Helix pomatia*). Our results concluded that 3dhE is the intimate precursor of E and results in the production of 20E in *P.Clarkii*.

1) H.Sonobe, M.Kamba, K.Ohta, M.Ikeda and Y.Naya, *Experientia*, **47**, 948(1991).