Ecdysteroids influence the circadian system timing ecdysis in the insect *Rhodnius prolixus* (Hemiptera)

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**Summary.** 20-hydroxyecdysone (20HE) injections induced transient delays in the time of ecdysis in *Rhodnius prolixus* reared in L/D cycles. Sustained phase delays in the ecdysis rhythm were revealed by transfer to constant dark during the scotophase following 20HE injection. The magnitude of the phase delays depended on the time in the L/D cycle at which 20HE was injected with major delays occurring at times when the endogenous titre is declining. Therefore the increases and decreases in the endogenous titre which are themselves timed in a circadian fashion may be involved in phase setting the ecdysis rhythm to the environmental cycle. Populations maintained in LL which are arrhythmic with respect to both ecdysteroid titres and ecdysis, can be induced to display gated ecdysis by injection of either 20HE or antiserum to ecdysteroids. Multiple injections of 20HE or antiserum are capable of inducing an ecdysis rhythm whose period (22.3 h) and gate location are very similar to that produced by altering the environmental cycle. Multiple manipulations of the endogenous titre of ecdysteroids can mimic the effects of L/D cycles on the timing of ecdysis. Ecdysis in *Rhodnius* may therefore be timed at least partially as a result of circadian timing of the ecdysteroid titre.

**Introduction**

A wide variety of events such as behaviour growth and development display endogenous forms of timing (see Saunders 1979; Rusak 1981). Blood levels of hormones may participate in the timing of such events (Menaker and Binkley 1981). The blood levels of steroids such as testosterone may act in concert with the external environment in order to regulate the release of hormones such as Lutenizing Hormone and Follicle Stimulating Hormone from the pituitary (see Turek and Ellis 1981). In insects, the steroid moultling hormones (ecdysteroids) can influence the excitability of the nervous system (Richter 1979; Ruegg et al. 1982; Steel and Davey 1985). In fact in *Manduca sexta*, ecdysteroids can delay both the release of and the responsiveness of the animal to the eclosion hormone which elicits ecdysial behaviour (Truman 1981; Truman et al. 1983). The nature of this response suggests that at least one action of ecdysteroids is to influence the endogenous timing system in *Manduca* (Truman 1981). Therefore, as in vertebrates, steroids such as ecdysteroids may influence or modulate behavioural or developmental responses to environmental inputs.

In the hemipteran, *Rhodnius prolixus*, the behaviours involved in shedding the old cuticle (ecdysis), are timed in an endogenous circadian fashion (Ampleford and Steel 1982a). In addition, the titre of ecdysteroids is also modulated by a circadian system (Ampleford and Steel 1985). Slama (1980) has proposed that the synchronization of ecdysis with morphogenetic changes in the epidermis may depend on ecdysteroids. In fact, the rate at which endocuticle is digested is influenced by ecdysteroids (Truman et al. 1983). The titre of ecdysteroids could provide the circadian system timing ecdysis with the cue for gate selection, indicating that development has progressed to the point at which ecdysis can be attempted. In addition, the rhythmic nature of the ecdysteroid titre in *Rhodnius* could influence timing properties of the circadian system controlling ecdysis.

In this paper, the evidence for an interaction between ecdysteroids and ecdysis is provided. Ecdysial...
ecdysteroids would appear to be able to act directly on the circadian system timing ecdysis influencing the phase of, and inducing an ecdysis rhythm. Thus circadian modulation of ecdysteroid would appear to be sufficient for the induction and maintenance of circadian timing of ecdysis. The interactions between ecdysteroids and ecdysis provide a unique opportunity to study the role of circadian systems in endocrine integration.

Materials and methods

Male fifth instar *Rhodnius prolixus* were allowed to entrain to the environmental cycle for at least one week prior to feeding. The moulting cycle was initiated by allowing the animals to feed on rabbit blood. After feeding, the animals were returned to the incubator (Percival) set at 28±0.5 °C. At this temperature, the imaginal ecdysis occurred 19 to 26 days after feeding. Ecdysis in *Rhodnius* can be easily recognized (see Ampleford and Steel 1982b). The time of ecdysis was monitored by time-lapse photography at 20 min intervals using Kodak 2415 film (see Ampleford and Steel 1982a). Time was plotted in Arbitrary Zeitgeber Time (AZT) where 0 represents the time of lights off. Statistical analysis of the data was by a non-parametric one-way analysis of variance (Kruskal and Wallis 1952).

Solutions of 20-hydroxyecdysone (20HE) were prepared by serial dilution and the concentration cross-checked by Radio Immuno Assay (RIA) (see Steel et al. 1982). Antiserum (AS) H3, ecdysone/20HE = 3 (see Gilbert et al. 1977) was diluted to a concentration where 1 μl of solution would bind 10 ng of 20HE by RIA. Control serum (rabbit serum, GIBCO) was diluted with distilled water to the same extent as the AS. Injections were performed through the membrane at the base of a metathoracic leg using a finely drawn calibrated micropipette. One μl doses of the test solutions were given on the day the first members of the population attempted ecdysis (Day 19 or 20 after feeding).

Results

*Rhodnius* maintained on 12L/12D cycles display gates for ecdysis between 7–13 AZT with the gate median or phase located at 10 AZT (Ampleford and Steel 1982a). In order to determine if 20HE could influence the time of ecdysis, populations maintained on 12L/12D were injected with 20 or 40 ng of 20HE or distilled water at 18 h AZT, and the time of ecdysis recorded. Injection of 20HE produced a significant delay in the time of ecdysis within the 24 h period following injection relative to the distilled water injected controls with more pronounced delays at the higher 40 ng dose (Fig. 1). The majority of the 20HE treated animals attempted ecdysis during the light portion of the cycle (12–24 AZT) at a time when ecdysis is not normally observed. In fact, ecdysis was observed up to and throughout the time of the expected gate in the second L/D cycle after injection. By the third cycle after injection, clear gates for ecdysis have returned displaying a median at 10 AZT. Therefore, 20HE can produce delays in the time of ecdysis within 24 h of injection.

The resumption of normal gated ecdysis following the initial 20HE induced delay may be due to the continued presence of the L/D cycle. If the L/D cycle was responsible for resetting or retraining the normal time of ecdysis, then injection followed by a transfer to constant dark (DD) should allow any injection induced delay in the