NONSPECIFIC EFFECTS OF LARGE DOSES OF 20-HYDROXYECDYSONE ON THE BEHAVIOR OF *AEDES AEGYPTI*

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ABSTRACT. Physiological amounts of 20-hydroxyecdysone (250 or 1000 pg) injected into female *Aedes aegypti* mosquitoes had no measurable effect on host-seeking, biting or oviposition behaviors. Larger doses of 0.5 and 1.0 μg reduced the proportion of mosquitoes which engaged in host-seeking and oviposition, but did not affect biting behavior. The host-seeking behavior of ovariectomized mosquitoes receiving a physiological dose of 20-hydroxyecdysone at 4–7 hr after a blood meal was not inhibited at 48 hr after the meal. It is suggested that large amounts of the hormone cause a traumatic, nonspecific behavioral inhibition.

Although mosquitoes are able to survive on a carbohydrate diet alone, the females of most species require a meal of blood as a source of protein for egg development. However, the vertebrates that provide this blood often display defensive behavior, which can be a significant factor in mosquito mortality (Edman et al. 1974). Consequently, it is not surprising to find mechanisms in the mosquito that inhibit host-seeking behavior once blood-feeding has occurred and oocyte maturation has been initiated, apparently to minimize the risks inherent in hematophagy.

Blood ingestion affects the subsequent host-seeking behavior of mosquitoes in at least 2 ways. An immediate inhibition of host-seeking results when the distention from a large blood meal activates abdominal stretch receptors (Klowden and Lea 1979b). This inhibition is short-lived, lasting only as long as the abdomen remains distended. A delayed, longer-lasting inhibition occurs in females taking blood meals large enough to sustain oogenesis. This second type of inhibition is first evident at 30 hr post-blood meal (pbm) in vitellogenic *Aedes aegypti* (Linnaeus), and usually lasts until after the completion of oviposition. A humoral substance present in the gravid female is involved in this second type of host-seeking inhibition (Klowden and Lea 1979a). Since this humoral inhibition of host-seeking accompanies oogenesis, and the mosquito ovary has been identified as an endocrine organ (Meola and Lea 1972, Hagedorn et al. 1975), it was originally suggested that the ovaries may be a source of a host-seeking inhibitor, but the possibility that other endocrine events associated with oogenesis may have an effect on behavior was not ruled out (Klowden and Lea 1979a). This influence of the ovaries was recently clarified; it appears that beginning at 10 hr pbm, they release a factor that initiates the release of the humoral inhibitor of host-seeking (Klowden 1981). The site of inhibitor release has not yet been identified.

The vitellogenic ovary of *Ae. aegypti* is also a source of ecdysteroids (Hagedorn et al. 1975). Beach (1979) proposed that the ovarian ecdysteroid present during vitellogenesis was responsible for inhibiting blood-feeding behavior in the gravid mosquito, perhaps by eliciting the production of a second factor that inhibited biting (Beach 1980). He demonstrated that *Anopheles freeborni* Aitken showed a reduced tendency to blood-feed beginning at 24 hr after the injection of from 0.5 to 5.0 μg of 20-hydroxyecdysone. There is no published information regarding the ecdysteroid content of *An. freeborni,* but these doses are approximately 10^4 to 10^5 times greater than

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the endogeneous hemolymph titer of 55 picograms (pg) in *Ae. aegypti* (Hanaoka and Hagedorn 1980).

As suggested in a preliminary report, 20-hydroxyecdysone injected in large doses may cause a traumatic, nonspecific inhibition of mosquito behavior which is unrelated to the inhibition of host-seeking that normally accompanies vitellogenesis (Klowden 1980). In this study, I present data showing that physiological doses of 20-hydroxyecdysone have no effect on the host-seeking, biting, or oviposition behavior of *Ae. aegypti*, and that large doses appear to inhibit behavior nonspecifically.

MATERIALS AND METHODS

MOSQUITO REARING. The UGAL strain of *Aedes aegypti* was used in all experiments. Larvae were reared on a standard diet (Klowden and Lea 1978), and adults were maintained at 27° and 80–90% RH under a 14:10 (light:dark) photoperiod. Ten percent sucrose was provided at all times except for 2 days prior to olfactometer testing, when water alone was available. All experiments were performed with 3- to 5-day-old mosquitoes. A rat anesthetized with ether was the source for all blood meals.

OLFACTOMETER. Mosquito host-seeking behavior was measured with the olfactometer described earlier (Klowden and Lea 1978), using a human host as a stimulus. A positive response to the host was scored if the mosquitoes flew upwind from a holding cage through a 1-m long flight section, and into a trap compartment. Testing took place during the first 3 hr of photophase. Groups of mosquitoes which showed a significantly reduced response to the host compared to controls were considered to be inhibited from host-seeking. In addition to testing host-seeking behavior, biting behavior was examined in some experiments by placing a container of mosquitoes directly on the host for 10 min and scoring for the ingestion of blood. The arcsine test for the equality of 2 percentages was used to determine the significance between the responses of experimental groups (Sokal and Rohlf 1969).

INJECTIONS AND SURGICAL MANIPULATIONS. To determine its effect on mosquito host-seeking behavior, 20-hydroxyecdysone (Rohto Pharmaceutical, Osaka, Japan) in 0.5 μl of insect saline (Ephrussi and Beadle 1936) was injected through the intersegmental abdominal membrane of female mosquitoes 1–2 days before they were tested in the olfactometer. In tests of oviposition behavior, mosquitoes were injected with 20-hydroxyecdysone at 24 hr pbm, isolated in individual cages, and given oviposition sites at 48 hr pbm. They were examined at 72 hr pbm for evidence of oviposited or retained eggs. Controls received 0.5 μl of saline.

Mosquitoes were ovariectomized under saline by removing each ovary with fine forceps introduced through the dorsal intersegmental membrane between the 5th and 6th abdominal segments. The wound was sealed with a small amount of paraffin.

RESULTS

EFFECT OF 20-HYDROXYECDYSONE ON HOST-SEEKING AND BITING BEHAVIOR. The host-seeking behavior of sugar-fed mosquitoes was not significantly inhibited by single physiological doses of 250 or 1000 pg of 20-hydroxyecdysone when tested at 24 or 48 hr post-injection. However, only about 40% of mosquitoes receiving larger doses of 0.5 and 1.0 μg responded to a host 24 hr after injection (Table 1). Although these large doses influenced mosquito host-seeking behavior, biting behavior, once the insects were placed on the host, was not affected (Table 2).

Beckemeyer and Lea (1978, 1980) demonstrated that 2 physiological doses of 20-hydroxyecdysone spaced 16 hr apart promoted the formation of secondary follicles in *Ae. aegypti*. This schedule apparently mimicked the 2 peaks of hormone observed by Hagedorn et al. (1975). To determine if 2 physiological doses

Table 1. Effect of exogenous ecdysterone on mosquito host-seeking behavior. Numbers followed by different superscripts within each experiment are significantly different ($p < 0.02$).

Amount of ecdysterone administered*	n	% of mosquitoes host-seeking
*Single injection, 24 hr olfactometer test***		
0***	118	86a
250 pg	61	77a
1000 pg	50	80a
0.5 μg	58	41b
1.0 μg	57	39b
*Single injection, 48 hr olfactometer test***		
0***	53	96a
250 pg	60	97a
1000 pg	30	100a
*Double injection, 24 hr olfactometer test***		
0***	30	90a
250 pg	30	90a

* Injected in 0.5 μl of insect saline.

** Single injection at 0 hr; double injections at 0 and 16 hr.

*** Saline-injected controls.

might also influence host-seeking, 2, 250 pg injections of 20-hydroxyecdysone, separated by 16 hr, were administered. The data in Table 1 show that host-seeking inhibition was not induced by this treatment.

Exogenous 20-hydroxyecdysone may be metabolized or excreted differently in sugar-fed mosquitoes than it is when the insects feed on blood. Therefore, in another experiment, ovariectomized mosquitoes were blood-fed and injected with 250 or 1000 pg of 20-hydroxyecdysone 4–7 hr later, and their host-seeking behavior was tested at 48 hr pbm. The ovaries were removed before the blood meal in order to eliminate any influence they may have in addition to their production of ecdysone. As shown in Table 3, 20-hydroxyecdysone administered to these ovariectomized, blood-fed mosquitioes failed to inhibit host-seeking behavior compared to controls injected with saline.

Table 2. Effect of exogenous ecdysterone on mosquito blood-feeding behavior.

Amount of ecdysterone administered*	n	% blood-feeding when placed directly on the host
0**	60	97
250 pg	31	90
0.5 μg	29	86
1.0 μg	28	96

* Injected in 0.5 μl of insect saline.

** Saline-injected controls.

EFFECT OF ECDYSTERONE ON OVIPOSITION BEHAVIOR. The administration of 0.5 or 1.0 μg of 20-hydroxyecdysone at 24 hr pbm significantly reduced the percentage of mosquitoes initiating oviposition by 72 hr pbm, compared to those insects receiving 250 pg of hormone or saline alone. The percentage of mosquitoes which did not oviposit their entire batch of eggs was significantly higher with 0.5 or 1.0 μg than in females given 250 pg or saline (Table 4). The viability of eggs from 20-hydroxyecdysone-injected females was comparable to that of saline-injected controls.

DISCUSSION

It has been suggested that in mosquitoes, ovarian ecdysteroids inhibit biting behavior (Beach 1979) or elicit the production of a second factor that acts as an inhibitor (Beach 1980). However,

Table 3. Host-seeking behavior at 48 hr pbm of mosquitoes ovariectomized before the blood meal and injected with ecdysterone 4–7 hrs pbm.

Amount of ecdysterone administered*	n	% of mosquitoes host-seeking
0**	47	68
250 pg	63	71
1000 pg	37	81

* Injected in 0.5 μl of insect saline.

** Saline-injected controls.

Table 4. Effect of exogenous ecdysterone on mosquito oviposition behavior. Numbers in each column followed by different superscripts are significantly different ($P < 0.02$).

Amount of ecdysterone administered*	n	% of mosquitoes ovipositing**	% of mosquitoes still retaining some eggs**
0***	57	88a	37a
250 pg	64	72a	58a
0.5 μg	62	39b	85b
1.0 μg	25	8c	100b

* Injected at 24 hr pbm in 0.5 μl of insect saline.

** Examined at 72 hr pbm.

*** Saline-injected controls.

these conclusions were based largely upon experiments in which mosquitoes were injected with doses of hormone that were well above physiological concentrations.

Hagedorn et al. (1975) showed that ecdysteroids reach a peak of approximately 275 pg by 18 hr pbm, and return to their low pre-blood meal levels by 48 hr pbm. It is unlikely that these endogeneous ecdysteroids directly inhibit the host-seeking behavior of *Ae. aegypti* females, because physiological doses of 20-hydroxyecdysone fail to affect this behavior (Tables 1 and 3). Also, hemolymph transfusions, from gravid donors at 48 hr pbm, inhibit host-seeking (Klowden and Lea 1979a) and reduce the sensitivity of lactic acid antennal receptors (Davis and Takahashi 1980), although ecdysteroid levels in donors are low at this time. It is also unlikely that ovarian ecdysteroids elicit the production of a second factor that inhibits mosquito host-seeking. The ovaries do initiate the release of a host-seeking inhibitor, but for the inhibition to be expressed, vitellogenic ovaries are only required for the first 10–12 hr pbm (Klowden 1981). This critical period precedes the rise in ecdysteroid titers reported by Hagedorn et al. (1975).

In contrast, large doses of 20-hydroxyecdysone not only inhibited host-seeking, but also affected oviposition behavior (Table 4). Beach (1980) also reported an effect of 20-hydroxyecdysone on oviposition in *An. freeborni*. He interpreted this as evidence that the hormone may naturally suppress both biting and oviposition, preventing an unnecessary blood meal from being taken and the premature deposition of eggs from occurring, and also inhibiting any extraneous activity during oogenesis. However, other species of mosquitoes which presumably also contain ecdysteroids during vitellogenesis still engage in other activities while maturing their eggs. For example, field studies demonstrate that several species feed on nectar throughout oogenesis (Magnarelli 1977, 1978; Nasci 1980). Furthermore, if host-seeking inhibition is merely one aspect of an overall behavioral inhibition normally induced by endogenous ecdysteroids, oviposition behavior, which commences at 48 hr pbm in *Ae. aegypti*, would not be possible, since it occurs during the period of maximum host-seeking inhibition that continues until at least 72 hr pbm, or until after eggs are laid (Klowden and Lea 1979a). Although gravid mosquitoes generally refrain from seeking a host, they do oviposit.

In contrast to host-seeking behavior, biting behavior, measured by placing the mosquito directly on the host, was not affected by the administration of even up to 1.0 μg of hormone. Fewer than 20% of gravid females, with endogenous ecdysteroids, will bite when placed on the host at 48 hr pbm (unpublished data), but 86–96% of mosquitoes still take a blood meal after receiving a large exogenous dose of 20-hydroxyecdysone (Table 2). Biting and blood-feeding are the terminal events in the sequence of behaviors which together comprise host-seeking behavior

(Kalmus and Hocking 1960). Roth (1951) has shown that antennectomized mosquitoes do not engage in host-seeking, but will bite when placed on the host, suggesting that different receptors may come into play during the various phases of host-seeking. Large amounts of exogenous ecdysterone appear to affect the initial behavior phases of searching for a host, but not the capacity to blood-feed once the host is located.

The reason that only large doses of 20-hydroxyecdysone affect behavior may be related to their rapid degradation and excretion (Ohtaki and Williams 1970, Moriyama et al. 1970). However, Beckemeyer and Lea (1978, 1980) showed that 2 physiological doses of 20-hydroxyecdysone, administered during a critical period, promoted formation of secondary follicles. This indicates that both these small doses withstood degradation to have their combined effect. As indicated in Table 1, 2 such doses did not affect host-seeking. Other possibilities requiring a large dose of hormone may relate to a higher threshold for responding to ecdysone in the target tissues of a non-blood-fed female compared to one which has blood-fed (Hanaoka and Hagedorn 1980), or to the physiological state of the insect affecting the rate at which circulating hormone is excreted (Hoffmann et al. 1974). However, physiological doses of 20-hydroxyecdysone, administered to ovariectomized, blood-fed mosquitoes, did not affect host-seeking (Table 3).

Only exogenous doses of ecdysteroids well above the physiological range stimulate vitellogenesis in non-blood-fed *Ae. aegypti.* Doane (1973) commented that although large doses of ecdysteroids often produce toxic effects of little developmental interest, abnormalities that may result can provide insights into the action of hormones. These insights into the mosquito system have been the subject of such speculation (Borovsky and Van Handel 1979, Hagedorn 1980, Kelly and Fuchs 1980, Borovsky 1981). Lea (1982) discussed the requirement for these high doses of 20-hydroxyecdysone, and demonstrated that they induced abnormal deposition of protein and lipid yolk in *Ae. aegypti,* and that physiological doses of 20-hydroxyecdysone, either by injection of hormone or implantation of ecdysone-secreting ovaries, were without effect.

Ecdysteroid titers do increase prior to the time that host-seeking inhibition occurs in *Ae. aegypti,* but this is hardly sufficient to imply a cause and effect relationship. Beach (1979) concluded that ecdysterone inhibits host-seeking; a reasonable alternative to this hypothesis is that large doses of a metabolically active hormone produce a nonspecific behavioral inhibition as a result of trauma.

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