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Termination of Adult Diapause by a Juvenile Hormone Analogue in the Bean Bug, *Riptortus clavatus*

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ABSTRACT — Diapausing adults of both sexes of *Riptortus clavatus* Thunberg were treated topically with a juvenile hormone analogue (JHA). JHA administered at 0.6 or 6 μg/insect induced yolk deposition in all females and complete ovarian development in some of them. It also evoked sexual behavior in most of the males at the same dosage. JHA was even effective in inducing yolk deposition in decapitated females at a dose of 6 μg/insect. Therefore, we concluded that adult diapause in *R. clavatus* is due to the cessation of JH secretion, and JHA can terminate diapause without intervention of the protocerebral neurosecretion.

INTRODUCTION

Diapause in adult insects is characterized by reproductive arrest and its hormonal mechanism has been demonstrated in many cases [1, 2]. The application of exogenous juvenile hormone (JH) or its analogue (JHA) terminates adult diapause and induces yolk deposition or oviposition in many species [3–11]. A few authors have reported that the frequency of mating increases in diapausing pairs when both sexes are treated with JH or JHA [3, 6, 8, 11].

The bean bug, *Riptortus clavatus* Thunberg (Heteroptera: Coreidae), exhibits a facultative adult diapause which is controlled by photoperiod [12]. The present paper describes the effect of a JHA on diapause termination in both sexes.

MATERIALS AND METHODS

Adults of *R. clavatus* were collected from legume fields in Kyoto. Their eggs were used for experiments performed at 25±1.5°C. Nymphs were reared by a method previously reported under a photoperiod with a 10-hr photophase and a 14-hr scotophase (10L–14D), which induced and maintained diapause in this species, or a diapause-preventing photoperiod of 16L–8D [12]. Two adults of the same sex were reared in a 200 ml plastic cup with soybeans and water.

JHA was obtained commercially as Manta® (Otsuka Chemicals Co.), an ethanol solution of methoprene (isopropyl [2E, 4E] 11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate). Seven days after adult e nergence, experimental groups of diapausing insects were treated topically on the abdomen with various doses of JHA in 5 μl ethanol. Control groups were either treated with ethanol or left untreated.

Seven days after JHA treatment, the diapause status, whether maintained or terminated, was examined. Statistical analyses were carried out between each experimental group and the control group, ethanol-treated and untreated groups combined, by Fisher's exact probability test.

Females were dissected and the developmental stages of their ovaries were classified as follows: (−) no yolk was deposited in oocytes; (+) light-blue colored yolk was deposited in oocytes; (++) mature eggs were ovulated into the oviduct. Individuals which had ovaries in stage (+) or (++) were considered to be diapause-terminated because light-blue yolk deposition never occurred in

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diapausing females [12]. JHA was also applied to some decapitated individuals in order to exclude the effects of the brain.

In *R. clavatus*, spermatozoa were found in the testes of the diapausing males, which were distinguished from the nondiapausing ones only by the lack of mating activity [12]. Assessment of diapause in males was therefore carried out upon induction of sexual behavior. Each experimental male was placed in an 80 ml plastic cup with a virgin nondiapausing female of the same age (14 days old) and the behavior of the male was observed for 30 min. In the sexual behavior of this species, the male invariably quivers while facing the female before mounting it. Therefore, quivering and copulation were employed as indices for mating activity in the male.

RESULTS

Females

Diapause was maintained in the control groups. Although application of JHA at 0.06 μg/insect was ineffective for diapause termination, it was effective at 0.2 μg/insect. JHA induced yolk deposition in all females and induced complete ovarian development in some of them at 0.6 or 6 μg/insect (Table 1). Eight male/female pairs treated with JHA at 6 μg/insect were reared. The females began to lay eggs 6–8 days after the JHA application. JHA was effective in inducing yolk deposition and exhibited a lethal effect at 6 μg/insect in diapausing females which were decapitated just before JHA application (Table 2).

TABLE 2. Effect of JHA on ovarian development in decapitated diapausing adults of *Riptortus clavatus* (10L–14D, 25°C)

Treatment	No. treated	No. died	Stage of ovaries+		
			−	+	++
Ethanol	20	1	19	0	0
6 μg JHA	20	8*	4	8***	0

+ Seven days after treatment. For −, +, ++, see text.
* $0.01<P<0.05$, *** $P<0.001$.

TABLE 3. Effect of JHA on the induction of sexual behavior in diapausing male adults of *Riptortus clavatus* (10L–14D, 25°C)

Treatment	No. treated	No. observed quivering+	No. observed copulating+
Control			
(Untreated)	20	2	0
(Ethanol)	20	3	1
0.06 μg JHA	20	7*	3
0.6 μg JHA	20	11**	4*
6 μg JHA	20	19***	13***

+ Response to nondiapausing females was examined seven days after treatment.
* $0.05<P<0.1$, ** $0.001<P<0.01$, *** $P<0.001$.

Males

All 20 of the male/female pairs reared under

TABLE 1. Effect of JHA on ovarian development in diapausing adults of *Riptortus clavatus* (10L–14D, 25°C)

Treatment	No. treated	Stage of ovaries+			% diapause terminated
		−	+	++	
Control					
(Untreated)	20	20	0	0	0
(Ethanol)	20	20	0	0	0
0.06μg JHA	20	20	0	0	0
0.2 μg JHA	20	14	6	0	30**
0.6 μg JHA	20	0	11	9	100***
6 μg JHA	20	0	4	16	100***

+ Seven days after treatment. For −, +, ++, see test.
** $0.001<P<0.01$, *** $P<0.001$.

16L–8D copulated successfully while the 20 male/female pairs reared under 10L–14D exhibited no sexual behavior whatsoever, 14 days after adult emergence. However, when paired with non-diapausing females, a small proportion of the males reared under 10L–14D in control groups quivered and one of them copulated successfully. JHA treatment induced sexual behavior in males even at 0.06 μg/insect, although the difference was not statistically significant. The proportion of individuals exhibiting sexual behavior increased with increasing dosage, although the dose-response relationship was less sharp than in females (Table 3).

DISCUSSION

It has been reported that inactivity of the corpora allata (CA) to secrete JH is of importance in controlling adult diapause in insects [1, 2]. Adult diapause is terminated by treatment with JH or JHA in many species, e.g., *Hypera postica* [3], *Oulema melanopus* [4], *Draeculacephala crassicornis* [5], *Eurygaster integriceps* [6], *Semiadalia undecimnotata* and *Coccinella septempunctata* [7], *Trypodendron lineatum* [8], *Drosophila grissea* [9], *Chrysopa* sp. [10] and *Leptinotarsa decemlineata* [10, 11]. However, in most of these species, a large dose of JH or JHA (50–100 μg/insect) was required to terminate diapause. For example, in *L. decemlineata*, methoprene has no effect on diapause termination at 100 μg/insect, although it inhibits pupal-adult metamorphosis at 0.001 μg/insect [10].

The protocerebral neurosecretion exerts not only an indirect role in activating the CA, but also direct action on yolk deposition in many species [1, 2]. The neurosecretory hormone in addition to JH has been suggested to be necessary for yolk deposition in diapausing adults of *Pterostichus nigrita* [13], *L. decemlineata* [14] and *Epilachna vigintioctopunctata* [15]. Schooneveld *et al.* [11] showed that a JH-induced termination of diapause in *L. decemlineata* is mediated through the neurosecretory system. A high dose JH or JHA may activate the protocerebral neurosecretory cells which affect ovarian development directly, in other species as well.

In *R. clavatus*, however, JHA effectively terminated diapause at a dose of 0.6 μg/insect in both sexes (Tables 1 and 3). Induction of sexual behavior in males isolated from females and treated with JHA confirms that JHA exerted its diapause-terminating effect on males themselves. It is intriguing that JHA induced yolk deposition even in decapitated females (Table 2). Therefore, we can conclude that adult diapause in *R. clavatus* is due to the inactivation of the CA, and JHA can terminate diapause without intervention of the protocerebral neurosecretion.

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