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SHORT- AND LONG-TERM EFFECTS OF PROALLATOTOXIN (ETHOXYPRECOCENE II) ON *RHODNIUS PROLIXUS* FEMALES

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Oogenesis and oviposition can be inhibited in female of Rhodnius prolixus *by means of short-therm experiment (first reproductive cycle) of a single dose of ethoxyprecocene II given by ingestion. The inhibition is dose-dependent as measured by oocyte growth, egg maturation and egg deposition. In a long-term experiment (second and third reproductive cycles) egg production and oogenesis can be partially or totally re-established by subsequent blood meals without ethoxyprecocene II. These findings suggest that in female* R. prolixus, *demage caused to* corpus allatum *by ethoxyprecocene II, in certain cases, is not irreversible.*

Key words: proallatotoxin – ethoxyprecocene II – *Rhodnius prolixus*

It is generally accepted that proallatotoxins, as precocenes and derivatives, induce juvenile hormone (JH) deficiency in several insect species through a mechanism of a selective destruction of the corpus allatum (CA). The cytotoxic action of precocenes is due to an oxidative activation by allatal monooxygenase enzymes forming, in situ, reactive intermediates, postulated as 3,4-epoxy-derivatives, which condense with macromolecular elements of the cytoplasm causing cell death (Bowers, 1982; 1983; Pratt et al., 1981).

The earliest evidence for the biological activity of precocenes was the induction of precocious metamorphosis in many species of sensitive insects. Consequently, a large number of papers has accumulated showing the action of proallatotoxins inducing adultoid formation (see Bowers, 1981; 1983). Another biological evidence for precocenes action is the induction of sterilization since the gonadotropic effect of JH in several insetcs is known. The chemical allatectomy caused by proallatotoxins, therefore, results in the sterilization of Hemiptera (Bowers et al., 1976; Tarrant et al., 1982), Orthoptera (Bergot et al., 1980; Pratt et al., 1980), Diptera (Bowers et al., 1976; Landers & Happ, 1980), and Coleoptera (Bowers et al., 1976; Rankin & Rankin, 1980).

However, in spite of all these studies, there is a lack of information concerning the persistance of sterilizing effects of precocenes (Bellés, et al., 1985). The present study, therefore, examines the effect of ethoxyprecocene II on the sterilization of adult female *Rhodnius prolixus*. In addition, we also follow the effect of this compound on the subsequent oviposition cycles, and show the partial or total recovery of the reproductive capability in these insects.

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MATERIALS AND METHODS

Insects – Male and female adults *Rhodnius prolixus* were used throughout this study. Insects were reared and maintained as previously described (Garcia et al., 1984). Experimental females were removed from the stock colony on the day of their last ecdysis and kept separately in glass jars. The females were fed and then mated for the first time.

Human blood and ethoxyprecocene II – Citrated human blood maintained at 4°C for some hours was used. Ethoxyprecocene II (EPII) diluted in ethanol was added to the blood meal to provide 1, 5 and 10 μg EPII/ml of blood. The insects were weighed immediately before and after being given their meals. Insects ingesting less than 140 mg of blood were discarded.

Oocytes measured – At various intervals after feeding both ovaries of at least five insects in each group, they were dissected under saline and the length of the seven terminal oocytes (T oocytes) was measured as described by Garcia et al., (1979). We also grouped the oocytes into three classes: (i) previtellogenic (those less than 0.4 mm long; (ii) vitellogenic (those greater than 0.4 mm but without chorion); (iii) mature eggs (those greater than 1.7 mm with the chorion).

Short- and long-term experiments – Short-term effects were checked until 20 days after the treatment. Therefore, only the first oogenic cycle was considered and the T oocyte growth, average number of eggs matured, and egg depo-

sition were measured. For long-term experiments only the egg production (which reflects the ovary maturation) in the second and third cycles of oviposition was considered. The experiments were ended 60 days after the treatment with EPII.

RESULTS

Short-term experiments – Each group of at least 10 insects received different doses of EPII. The doses (μg EPII/ml of blood) were: 1.0; 5.0; and 10.0. A control group received blood with the solvent only. Egg production as determined during 20 days of the first cycle of oviposition following one feeding session is shown in Table I. EPII decreased egg production in *Rhodnius* at all three concentrations used. The treated insects, even at the lowest dose of EPII given, never reached the rate of oviposition attained by the control females (Table I). For a period of 20 days, control females produced an average of 22 eggs. The EPII-treated groups produced 10 eggs per female in the group receiving 1.0 μg EPII/ml of blood, or even less in the other treated groups (Table I). The eggs laid by treated females were not affected in their viability.

Oocyte growth of control mated females along the first vitellogenic cycle is shown in Table II. Yolk deposition begins, and as yolk accumulates, the T oocytes enlarge. Fifteen to twenty days after ecdysis and just before having their first blood meal as adults, the T oocytes of normal females averaged 0.41 ± 0.04 mm; 4 and 6 days later these oocytes were 1.10 ± 0.08 and 1.51 ± 0.11 mm in length respectively (Table II). The effects of EPII on the vitellogenesis is also shown in Table II. The ingestion of EPII prevented yolk deposition during the six days after feeding, and the T oocytes did not show, for the groups receiving 5.0 and 10.0 μg EPII/ml of blood, any significant increase, and they practically never attained the stage of vitellogenesis. The group treated with 1.0 μg EPII/ml of blood had small increase in the T oocytes, however, they never reached the same rate of vitellogenesis attained by the control group (Table II).

Table III shows the percentage of yolky-filled oocytes and number of mature eggs attained by the control and treated groups on the 7th day after feeding (one day before initiating oviposition in the control group). It shows that there was vitellogenesis in 95% of the T oocytes and 6.4 ± 0.5 eggs were mature in the control females. On the other hand, yolky T oocytes and the average of mature eggs were about 50% or less in the EPII-treated groups.

Long-term experiments – In order to study the effects of EPII on the long-term sterilization, we observed the production of eggs in the treated-female groups after the second and third oviposition cycles, i.e., after treated females had received two fresh blood meals without EPII. The results are summarized in Table IV. We observed that the decrease of oviposition caused by ingested EPII could be partially or totally reversed in all doses of the compound by a feeding without it. This observation was specially evident comparing the results of the group treated with 1.0 and 5.0 μg EPII/ml with the control in the second cycle (Table IV) and with the same groups on the first reproductive cycle. In the third cycle of oviposition only the group receiving 10.0 μg EPII/ml still presnted a drastic reduction in the production of eggs.

Finally, it is worth mentioning that longevity of EPII-treated females was clearly lower in the highest dose of the compound in comparison with controls. In the other groups, the mortality was practically the same as that of the control (not shown).

DISCUSSION

According to the present findings and to previous studies on CA of in vivo precocene-treated insects (Unnithan et al., 1977; Pener et al., 1978; Schooneveld, 1979; Azambuja et al., 1981), it can be suggested that EPII induced a broad variety of changes on the CA which resulted in the absence of JH (Muller et al., 1979; Pratt & Bowers, 1977). Consenquently, precocenes cause antigonadotropic effeet in several

TABLE I

Cumulative numbers of eggs laid during 20 days in the first cycle of oviposition by control, and by the experimental females receiving ethoxyprecocene II

Treatments (μg of EPII/ml of blood)	eggs/female/day *
Control	1.10 ± 0.25
1.0	0.51 ± 0.12
5.0	0.31 ± 0.05
10.0	0.25 ± 0.02

* Each number is the man ± S. E. of at least 10 females.

TABLE II

Effect of ethoxyprecocene II on the lenght of T oocytes, 0-6 days following feeding

Treatments (μg of EPII/ml)	Days after feeding	T oocytes (mm)*
Control	0	0.41 ± 0.04
	2	0.76 ± 0.06
	4	1.10 ± 0.08
	6	1.51 ± 0.11
1.0	2	0.36 ± 0.02
	4	0.55 ± 0.08
	6	0.78 ± 0.09
5.0	2	0.36 ± 0.04
	4	0.40 ± 0.06
	6	0.41 ± 0.03
10.0	2	0.32 ± 0.01
	4	0.30 ± 0.00
	6	0.31 ± 0.01

* Each value represents the mean ± S. E. of the T oocytes present in both ovaries of the 5 females. The experiment began 15-20 days after ecdysis.

TABLE III

Effect of ethoxyprecocene II on vitellogenesis on 7th day after feeding

Treatments (μg of EPII/ml)	Percentage of vitellogenic oocytes	Average number of eggs matured by females *
Control	95	6.4 ± 0.5
1.0	75	3.3 ± 0.4
5.0	45	0.8 ± 0.3
10.0	25	0.4 ± 0.1

* Each value represents the mean ± S.E. of 5 insects.

TABLE IV

Average rate of egg production during the second and third cycles of oviposition in females that received different doses of ethoxyprecocene II in the first blood meal, followed by two sessions of feeding blood without the compound

Treatments (μg of EPII/ml)	eggs/ female/ day *	
	cycle 2 **	cycle 3 **
Control	1.38 ± 0.20	1.05 ± 0.10
1.0	0.98 ± 0.35	1.15 ± 0.25
5.0	0.65 ± 0.20	0.85 ± 0.36
10.0	0.38 ± 0.20	0.55 ± 0.28

* Each number is the mean ± S. E. of at least 7 females.
** After 2nd and 3rd blood meals without ethoxyprecocene II.

species of insects (see Bowers, 1981; 1983). In females *R. prolixus* a partial support of this hypothesis is that the application of JH caused a reversal of the inhibition of oogenesis in the insect that received EPII (Garcia & Azambuja, 1985).

In short-term experiments (first cycle of oviposition), EPII inhibited oocyte growth and egg maturation confirming previous data from this laboratory (Garcia & Azambuja, 1985). However, comparison of the results with those obtained from long-term experiments, indicates that a high percentage of insects is able to recover their reproductive capability. This clearly suggests that damage of CA induced by EPII was reversible in several cases. In this context, it is important to point out that similar conclusion was reported by Feyereisen et al. (1981) and Bellés et al. (1985) using two species of Dictyoptera, *Diploptera punctada* and *Blatella germanica,* respectively. Contradictory data were reported by Unnithan et al. (1977) in adult *Oncopeltus fasciatus,* and Schooneveld (1979) in nymphs of *Locusta migratoria.* However, no studies on the effect of precocenes on CA in long-term experiments have been done. Bellés et al. (1985), using indirect measures, suggested that the damage caused by precocene on the CA did not appear to be irreversible. Feyereisen et al. (1981) to explain their results, suggested that precocenes could affect similar structures of CA cells of different species. They also pointed out that secondary responses of the cells may not be necessarily the same. This suggestion, in addition with factors like precocene metabolism, treatment by ingestion and CA intrinsic susceptibility, might explain the reversal effect of EPII presented in this paper. We did find, however, an irreversible sterilization, when female *Rhodnius prolixus* received EPII by continuous contact treatment (Azambuja & Garcia, unpublished data).

RESUMO

Efeitos a curto e longo prazos, da proalatotoxina (Etoxiprecoceno II) em fêmeas de *Rhodnius prolixus* – A ovogênese e a postura de ovos pode ser inibida, durante o primeiro ciclo reprodutivo de *Rhodnius prolixus,* por um único tratamento com etoxiprecoceno II. Esta inibição, se medida pelo crescimento dos ovócitos, maturação de ovos e ovipostura, depende da dose do composto utilizado. A produção de ovos, no entanto, pode ser parcial ou totalmente restabelecida após dois subseqüentes repastos de sangue sem a droga. Estes dados sugerem que a ação do etoxiprecoceno II sobre o *corpus allatum* não é irreversível em fêmeas de *Rhodnius prolixus.*

Palavras-chave: etoxiprecoceno II – *Rodhnius prolixus* – proalatotoxina

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