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# EFFECTS OF PROALLATOTOXINS (PRECOCENES) ON THE DEVELOPMENT AND REPRODUCTION OF *RHODNIUS PROLIXUS:* SOME DATA

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*Proallatotoxins, and particularly precocenes, are exceptionally promising models for studying* Rhodnius prolixus *physiology and for comparison with other natural compounds with anti-hormonal activities. Effects of precocenes on feeding, development and reproduction of* R. prolixus *are being detailed. The precocenes reveal significant effects on feeding, moulting cycle (inducing precocious metamorphosis and ecdysial stasis), and reproduction of these insects. The mechanism of action of proallatotoxins was discussed based on the* corpus allatum *cytotoxic effect and on the ecdysteroid biosynthesis in prothoracic glands and ovaries. Further studies of these compounds on* R. prolixus *are need and will hopefully reveal other unexplored points regarding the action of the proallatotoxins on insects.*

Key words: *Rhodnius prolixus* – precocene – proallatotoxin

Proallatotoxins as precocenes (precocene I, PI; precocene II, PII), are naturally occuring compounds found in several species of plants in the genus *Ageratum* (Bowers, 1976; Bowers et al., 1976). Their applications in many paurometabolous species cause a variety of behavioral, physiological and biochemical changes including precocious metamorphosis of the immature stages and sterilization of adult females. Since insect hormones are known to control larval development and egg production these compounds were immediately considered to have an anti-hormonal action, and particularly an anti-juvenile hormonal effect. Proallatotoxins usually are cytotoxic to the insect secretory cells of the *corpus allatum*, thereby eliminating the production of juvenile hormone (Bowers, 1976, 1981, 1982). Thus, the interruption of normal larval development to produce precocious adults and the sterilization of adult females has been reported in several hemimetabolous species, whereas in holometabolous species only a few findings on sterilization, but not induction of adultoids have been reported (Bowers et al., 1976; Pener et al., 1978). Even in sensitive groups of insects large difference are seen between species as regards the efficiency of precocenes (Bowers, 1988).

The mechanism of action of these compounds is now moderately explained, and will be discussed in detail in the present paper.

## THE *RHODNIUS* BIOASSAY

Larvae and adults of the hemipteran hematophagous, *R. prolixus* were reared and maintained using an artificial feeding apparatus described previously (Garcia et al., 1984). This insect is relatively high sensitive to feeding deterrents and insect growth inhibitors. In several cases both effects come up by the same natural chemical. Therefore, fourth-instar larvae and adult females of *R. prolixus* came out to be a suitable animal model and a very powerful tool for this purpose. For routine uses, the insects were starved and then fed on blood containing the compounds, or alternatively the active compounds were applied topically. The intake of blood ingested and the compound doses were easily determinated by the difference in the body weight measured before and after feeding (Azambuja et al., 1982).

## ANTIFEEDING EFFECTS

Usually, unfed insects present disturbance in the endocrine organs being necessary thus to distinguish what is antifeedant and anti-hormonal activities of the compounds. Slama (1978), for example, speculated that the effects of precocenes on the development and repro-

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duction of insects might be due to an antifeedant action of these compounds. Reduced feeding by *Rhodnius* treated with PII prompted us to investigate whether the starvation could account for the observed effects of these chemicals (Azambuja et al., 1981b). We found that feeding of *R. prolixus* was significantly diminished when PII was added either to blood meal or to artificial diet ($ED_{50}$ of 48 $\mu$g/ml and 43 $\mu$g/ml, respectively). In contrast, PI and the synthetic ethoxy-PII (EPII) only showed a modest decrease in feeding ($ED_{50}$ of 260 $\mu$g/ml and 140 $\mu$g/ml, respectively). Interestingly, EPII has clearly more potent effects on *Rhodnius* development than PII (Azambuja et al., 1982). These results emphasized that the antifeedant and anti-hormonal actions of precocenes are distinct and especially that these compounds have a true anti-hormonal effect on this species.

## EFFECTS ON IMMATURE STAGES

The first evidence for the biological activity of precocenes was the alteration of the moulting cycle and induction of precocious metamorphosis in sensitive insects (Bowers, 1976; Bowers et al., 1976).

*Precocious metamorphosis (induction of adultoids)* – Topical, feeding and continuous contact treatments with PII and EPII, in different proportions, were able to induce adultoid formation and caused moulting delay in all stages of development in *R. prolixus* (Tarrant & Cupp, 1978; Tarrant et al., 1982; Azambuja et al., 1981a, b; Garcia et al., 1984). A significant correlation between the prolongation of the moulting cycle and morphogenetic adultoid characteristics was observed by oral treatments with PII. *Rhodnius* adultoids with immature characters were obtained in the first days of the ecdysis, and from those insects which presented ecdysis arresting emerged adultoids with imaginal characteristics (Azambuja et al., 1981b). The insects with slight adult characteristics were able to feed and initiate the next ecdysis, and those with distinct imaginal characters were short-lived and did not feed even in condition of repeated opportunities (Azambuja et al., 1981a, b).

*Moulting delay and ecdysial stasis* – Despite the morphogenetic effects of precocenes in *R. prolixus* they also caused delay in the moult, i. e., many treated larvae failed to continue ecdysis remaining as larvae until death (Tarrant & Cupp, 1978; Azambuja et al., 1981a, b; Garcia et al., 1984). Although PI had no apparent effect on the induction of precocious metamorphosis as occurred with PII and EPII, the action of these three compounds on moulting stasis by topical and oral treatments are clear. The highest production of ecdysial stasis (38% of the treated insects) was obtained by oral treatment (Garcia & Azambuja, 1987). It seems therefore that precocenes were involved in the disruption of processes related to the ecdysteroid control. The earliest evidence for such fact was that ecdysone given orally counteracted the moulting arrest and reduced the ecdysial stasis as induced by PII and EPII (Azambuja et al., 1981a, b; Garcia et al., 1984). These findings suggested that precocenes induced cessation of juvenile hormone release, that somehow indirectely depressed ecdysone production. In support to this hypothesis it has been shown that allatectomy influenced the ecdysteroid-dependent development of *R. prolixus* larvae (Garcia et al., 1987a). However, recent findings by Garcia et al. (1987b, 1988) indicated that although prothoracic glands cultured *in vitro* produced ecdysteroid spontaneously, when they were incubated with EPII ecdysteroid production was prevented. These data suggest that proallatotoxins have a potential use as a further tool in insect control, by means of the inhibition of ecdysone biosynthesis.

## EFFECTS ON REPRODUCTION

In many insects, including *R. prolixus* (Davey, 1980), it has been described that surgical ablation of the *corpus allatum* is followed by sterilization. These findings clearly show the importance of juvenile hormone as a gonadotropic hormone. Similarly, the effect of proallatotoxins results in the sterilization of *R. prolixus* (Azambuja & Garcia, 1987). Assessment of the antigonadotropic activity of PI, PII and EPII is easily accomplished by topical and oral treatments of adult females of *R. prolixus*, with subsequent examination of ovarian development (lenght of terminal oocytes), vitellogenesis, egg production and ecdysteroid release from ovaries (Garcia et al., 1987a). In some examples a single treatment with proallatotoxins provides drastic sterilization. In other instances insect sterilization can be obtained by a brief treatment, but the destruction of the *corpus allatum* may be partial and egg production may later supervene in the absence of additional

exposure to precocenes (Azambuja & Garcia, 1987). We observed therefore that oogenesis as well as egg maturation and production can be drastically inhibited by means of short-term experiment (first reproductive cycle) of a single dose of EPII given by ingestion. However, in a long-term experiment (second and thrid reproductive cycles) the egg production and oogenesis can be partially or totally re-established by subsequent blood meals without the compound. Precocene-induced sterilization can be reversed in virtually all insects by a combined or subsequent treatment with juvenille hormone III (Garcia et al., 1987b).

Although ecdysone play a role in vitellogenesis of some insects (Hagedorn, 1983), virtually nothing is known about this hormone affecting vitellogenin synthesis in *R. prolixus* (Davey, 1980). Mature ovaries of *Rhodnius* produce ecdysteroids (Ruegg et al., 1981; Garcia et al., 1987b; Feder et al., 1988). It has been shown that EPII decline the titers of ecdysteroid in the hemolymph of *R. prolixus* adult females (Garcia et al., 1987b). *In vitro* synthesis of ovarian ecdysteroids is inhibited in insects receiving EPII-treatment and/or in normal ovaries incubated in a medium containing EPII (Garcia et al., 1987b). Nevertheless, the relevance of these data need to be deeperly investigated.

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REFERENCES

AZAMBUJA, P.; BOWERS, W. S.; RIBEIRO, J. M. C. & GARCIA, E. S., 1982. Antifeedant activity of precocenes and analog on *Rhodnius prolixus*. *Experientia, 38:* 1054-1055.

AZAMBUJA, P. & GARCIA, E. S., 1987. Short- and long-term effects of proallatotoxin (ethoxyprecocene II) on *Rhodnius prolixus* females. *Mem. Inst. Oswaldo Cruz, 82:* 247-251.

AZAMBUJA, P.; GARCIA, E. S. & FURTADO, A. F., 1981a. Action du precocene II sur le retard de la mue et de la metamorphose chez *Rhodnius prolixus*. *C. R. Acad. Sci. Paris, 292:* 1173-1175.

AZAMBUJA, P.; GARCIA, E. S. & RIBEIRO, J. M. C., 1981b. Effects of ecdysone on the metamorphosis and ecdysis prevention induced by precocene II in *Rhodnius prolixus*. *Gen. Comp. Endocrinol., 45:* 100-104.

BOWERS, W. S., 1976. Discovery of insect anti-allatotropins, p. 394-408. In L. I. Gilbert, *The Juvenile Hormones*. Plenum, New York.

BOWERS, W. S., 1981. How anti-juvenile hormones work. *Am. Zool., 21:* 737-742.

BOWERS, W. S., 1982. Toxicology of the precocenes, p. 403-427. In J. R. Coats, *Insecticide Mode of Action*. Academic Press, New York.

BOWERS, W. S., 1988. Cytotoxic defensive strategies of plants: chemistry and mode of action, p. 19-26. In F. Sehnal; A. Zabza & D. L. Delinger (eds), *Endocrinological Frontiers in Physiological Insect Ecology*.

BOWERS, W. S.; OHTA, T.; CLEERE, J. S. & MARSELLA, P. A., 1976. Discovery of insect anti-juvenile hormones in plants. *Science, 193:* 542-547.

DAVEY, K. G., 1980. The physiology of reproduction in *Rhodnius* and other insects: some questions, p. 325-344. In Locke, M. & Smith, D. S. (eds), *Insects Biology in the Future*. Academic Press, New York.

FEDER, D.; VALLE, D.; REMBOLD, H. & GARCIA, E. S., 1988. Azadirachtin-induced sterilization in mature females of *Rhodnius prolixus*. *Z. Naturforsch., 43c:* 908-913.

GARCIA, E. S. & AZAMBUJA, P., 1987. Induction of ecdysial stasis with structurally analogous proallatotoxins, p. 109-112. In R. Greenhalgh & T. R. Roberts (eds), *Pesticide Science and Biotechnology*. Blackwell Sci. Publ., Boston.

GARCIA, E. S.; AZAMBUJA, P. & BOWERS, W. S., 1984. Comparison of structurally analogous allatotoxins on the molting and morphogenesis of *Rhodnius prolixus*, and the reversal of ecdysial stasis by ecdysone. *Arch. Insect Biochem. Physiol., 1:* 367-373.

GARCIA, E. S.; AZAMBUJA, P.; FEDER, D. & BOWERS, W. S., 1988. Inhibition of ecdysteroid production in nymphs of *Rhodnius prolixus* treated with ethoxyprecocene II. *Arch. Insect Biochem. Physiol., 8:* 127-134.

GARCIA, E. S.; FEDER, D.; GOMES, J. E. P. L. & AZAMBUJA, P., 1987b. Effects of precocene and azadirachtin in *Rhodnius prolixus:* some data on development and reproduction. *Mem. Inst. Oswaldo Cruz, 82* (Suppl. III): 67-73.

GARCIA, E. S.; FURTADO, A. F. & AZAMBUJA, P., 1987a. Effect of allatectomy on ecdysteroid-dependent development of *Rhodnius prolixus* larvae. *J. Insect Physiol., 33:* 729-732.

HAGEDORN, H. H., 1983. The role of ecdysteroids in the adult insect, p. 271-304. In R. G. H. Downer & H. Laufer (eds), *Endocrinology of Insects*. Alan R. Liss, New York.

RUEGG, R. P.; KRIGER, F. L.; DAVEY, K. G. & STEEL, C. G. H., 1981. Ovarian ecdysone elicits release of a myotropic ovulation in *Rhodnius*. *Int. J. Invertebr. Rep., 3:* 357-361.

PENER, M. P.; ORSHAN, L. & DE WILD, J., 1978. Precocenes cause atrophy of *corpora allata* in *Locusta migratoria*. *Nature, 272:* 350-354.

TARRANT, C. A. & CUPP, E. W., 1978. Morphogenetic effects of precocene II on the immature stages of *Rhodnius prolixus*. *Trans. R. Soc. Trop. Med. Hyg., 72:* 666-669.

TARRANT, C. A.; CUPP, E. W. & BOWERS, W. S., 1982. Effects of precocene II on reproduction and development of triatomines bugs. *Am. J. Trop. Med. Hyg., 3:* 416-420.