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In vitro inactivation of corpora allata of the bug *Oncopeltus fasciatus* by precocene II

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Summary. Corpora allata from *Oncopeltus fasciatus* incubated in vitro in medium containing $10^{-5.35}$ M (1 µg/ml) of precocene II lose their ability to secrete juvenile hormone when reimplanted into last instar larvae.

Precocenes were the first substances discovered with antiallatotropic activity in insects[2]. Larvae of the large milkweed bug, *O. fasciatus,* reared in contact with sublethal doses of precocene II, undergo a precocious metamorphosis and moult into diminutive forms with adultoid characters. Freshly hatched females exposed to precocene remain sterile for the rest of their life. Their corpora allata (CA) are inactive[3] and do not regain their activity when transplanted into last instar larvae[4]. This indicates that precocene somehow inactivates the CA and thus blocks biosynthesis of juvenile hormone (JH). Submicroscopical changes and degeneration of CA were observed in treated *O. fasciatus*[5] and *Locusta migratoria*[6].

Precocene was found to be a modest inhibitor of JH biosynthesis in cultures of CA from cockroaches[7,8]. On the other hand, the morphogenetic and sterilant effects in these insects are negligible (Masner, unpublished results). We have examined the in vitro effect of precocene on the CA of *O. fasciatus* where the in vivo activity is very high.

Material and methods. A sunflower strain of the large milkweed bug, *O. fasciatus,* was reared at 18 h daily illumination, 30 °C and 60% relative humidity[9]. Fertile (copulating) females containing ripe eggs were used as donors of corpora allata-cardiaca complexes (CA). The animals were surface sterilized in a 3% sodium hypochlorite solution and dissected in sterile Ringer solution at an airflow sterile bench.

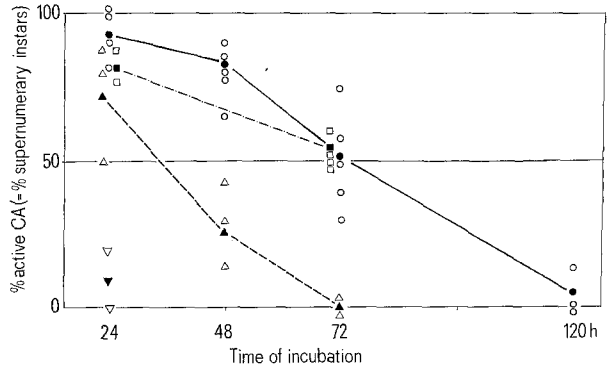
CA were incubated for 1-10 days at 30 °C in Leighton tissue culture glass tubes (Bellco, Biological Glassware, USA). Fully synthetic medium S_{20F}[10] or Shields medium[11] without serum or haemolymph were used (1 ml or 0.18 ml per flask). All solutions were filter-sterilized. The cultures which showed contamination (colour change of phenol red indicator) were discarded. Precocene II (6,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran)[2] or the chemically closely related β-asarone ((Z)2,4,5-trimethoxy-1-propenyl benzene)[12] were dissolved in absolute ethanol and added to the culture medium. An equivalent amount of ethanol (concentration $\leqslant 10^{-3.6}$ M) was added to the control medium.

The activity of the CA was assayed by transplantation of the gland into young fifth (last) instar larvae anaesthetized by submersion in water[13]. The outcome of the ensuing ecdysis of the host provided evidence about the morphogenetically active JH output of the gland. The percentage of supernumerary instars was considered to be proportional to the percentage of active glands. The inactive CA allowed normal adult development of the hosts[4].

Results. In the first series of experiments CA were incubated in the medium S_{20F}. More than 90% of glands incubated

Percent of morphogenetically active CA after incubation for 5 or 10 days in Shields and Sang's medium, to which precocene II, β-asarone or equivalent of ethanol (untreated) was added

Substance	Dose 10^{-x} M	Percent of active CA after incubation for 5 days	n	10 days	n
Precocene II	4.35	13	16		
	5.35	64	28	20	20
	6	89	9		
β-Asarone	4.32	100	19		
Untreated	-	94	18	90	33



Percent of morphogenetically active CA after incubation for 24-120 h in medium S_{20F}. The number of transplantations performed in 2-5 series (empty symbols) varies between 17 and 45. The full symbols indicate the mean values. Incubation in control medium (●——●), in medium with $10^{-4.35}$ M (▼), $10^{-5.35}$ M (▲——▲) and 10^{-6} M (■–·–■) of precocene II.

in the untreated medium for 24 h were active (figure). With prolonged incubation time the activity of the CA declined. After 72 h about one half of the CA were inactive and after 120 h all the glands were devoid of activity.

Almost all CA incubated for 24 h in the medium containing precocene at a concentration of $10^{-4.35}$ M were inactive. The same incubation time with a precocene concentration of $10^{-5.35}$ M reduced the activity only slightly. However, the incubation for 48 h at the latter concentration inactivated 69% and the still longer incubation for 72 h inactivated all the glands. These 2 values differ with high significance from the control data in Fisher's test[14]. The lower concentration of 10^{-6} M did not affect the activity after 120 h of incubation.

In the second series of experiments CA were incubated in a medium recently described by Shields and Sang[11]. In the control medium 90% of the glands remained active even after 10 days of incubation (table). In the medium containing precocene at the concentration $10^{-4.35}$ M only 10% of the glands were active after 5 days. Similar results were obtained after 10 days incubation with a concentration 10 times lower. On the other hand, in the medium containing β-asarone at the high concentration of $10^{-4.32}$ M all the glands remained active after 5 days incubation.

Discussion. CA taken from fertile females of *O. fasciatus* and cultivated in vitro for a considerable period of time remain morphogenetically active. This activity declines faster in Landureau's medium S_{20F}[10] than in Shield and Sang's medium[11]. Addition of precocene II to either medium causes a rapid decline in activity of the cultivated glands. These glands do not regain their activity when transplanted into untreated last instar larvae. β-asarone[12] is chemically related to precocene yet it lacks any morphogenetic activity when applied in sublethal dosages to *O. fasciatus* in vivo (P.J. Müller, unpublished results). This compound was also found to be inactive in our in vitro system at a concentration 10 times higher than the active dose of precocene. The results indicate firstly that precocene acts directly on the CA and inhibits its morphogenetic activity and secondly that the inhibitory effect is specific to precocenes.

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Effect of testosterone on the hypothalamic-pituitary-gonadal axis of pinealectomized and pineal-intact male rats[1]

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Summary. Previous studies indicate that steroid hormones alter pineal biochemistry, and it has been suggested that at least part of the negative feedback effect of steroid hormones on pituitary gonadotropin release may be mediated by the pineal gland. In this study, pinealectomy did not alter the inhibitory effect of testosterone on neuroendocrine-gonadal activity in the male rat, suggesting that the pineal gland does not mediate the response of the rat hypothalamic-pituitary axis to testosterone.

The administration of either gonadal steroid hormones or the pineal product, melatonin, to rats inhibits neuroendocrine-gonadal activity[2,3]. A number of reports indicate that steroid hormones can modulate pineal melatonin synthesis[4,5] and this has led to the suggestion that the pineal gland may mediate, in part, the negative feedback effect of gonadal steroids on hypothalamic-pituitary activity[6]. Evidence for an interaction between melatonin and steroid hormones in male rats is found in the observation that castration leads to a decrease in pineal hydroxyindole-O-methyltransferase activity (HIOMT), the enzyme which converts N-acetyl serotonin to melatonin, while the administration of testosterone propionate stimulates pineal HIOMT activity[7]. Similar effects are found following ovariectomy and estrogen treatment in female rats[8,9]. If the inhibitory action of steroid hormones on pituitary gonadotropin release is indeed modulated by the pineal gland, then pinealectomy should alter the response of the neuroendocrine-gonadal axis to steroid treatment. In this study, we sought to test the hypothesis that the pineal gland mediates the action of steroid hormones, by determining if pinealectomy alters the response of the hypothalamic-pituitary-gonadal axis to the negative feedback effect of testosterone in intact and castrated male rats.

Mature male albino rats weighing 180–200 g were purchased from ARS Sprague-Dawley, Madison, Wisconsin. The animals were maintained throughout the studies in a room provided with 14 h of light per day (LD 14/10), and they were housed 4–5 per cage with food (Purina rat chow) and water provided ad libitum. After an acclimation period of 2–3 weeks, the animals were either sham-pinealectomized (sham-Px) or pinealectomized (Px) according to the method of Hoffman and Reiter[10]. In 1 study, rats were then implanted s.c. with either empty or testosterone-filled silastic capsules that were 10 or 20 mm long (8/group). In a 2nd study, sham-Px and Px animals were castrated and 9–11 days post-castration they were implanted with either empty or testosterone capsules that were 10 or 20 mm long (8/group). 60 days following capsule implantation, the animals were sacrificed by decapitation, blood was collected, and the testes were removed and weighed.