Precocene inhibition of vitellogenesis in *Drosophila melanogaster*[1]

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Summary. A single topical application of precocene I or precocene II inhibits the normal process of vitellogenesis in *Drosophila melanogaster*. This effect is partly prevented by treatment with JH-11 or spontaneously reversible with time.

Hormone antagonists and endocrine mutants facilitate analysis of endocrine regulation of development. Precocenes, chromene derivatives recently isolated from bedding plants[2], are juvenile hormone antagonists which induce precocious metamorphosis[2,3] or produce anti-gonadotrophic effects[2] in certain insect species. We report herein the 1st demonstration that precocenes are anti-juvenile hormones in *Drosophila melanogaster*. Both wild-type flies treated with precocene and *apterous-4* (*ap*^{4}, 2-55.2) mutant flies, which are deficient in juvenile hormone for vitellogenesis[4], can be rescued by exogenous juvenile hormone. Finally, we report the 1st demonstration that precocene effects can be reversed spontaneously as the flies age.

Vitellogenesis in *Drosophila* requires juvenile hormone, produced normally by the corpora allata[4–6]. Vitellogenesis does not occur in abdomens of adult female *Drosophila*, which have been isolated before a critical period, but Handler and Postlethwait rescued such abdomens by administration of an exogenous juvenile hormone analogue or implantation of corpora allata[7].

We have applied precocenes-1 and -2 to the abdomens of intact newly-ecdysed virgin *Oregon-R-C* wild-type *(Ore-R-C)* female adult *Drosophila* and inspected their ovaries 48 h thereafter. Both precocenes dramatically inhibited oocyte growth (figure 1,b) relative to controls (figure 1,a). The ovaries of the precocene-treated *Ore-R-C* previtellogenic females resembled those of *ap*^{4} homozygotes (figure 1,c). Increased fat body was characteristic of both precocene-treated *Ore-R-C* and *ap*^{4} females.

The inhibition of oocyte growth in the previtellogenic *Ore-R-C* females might be explained either by a general toxic effect of precocene on *Drosophila* or by an anti-juvenile hormone action of precocene. To distinguish between these alternatives, we applied juvenile hormone in combination with precocene to newly-emerged *Ore-R-C* females. Significant oocyte growth followed this juvenile hormone rescue

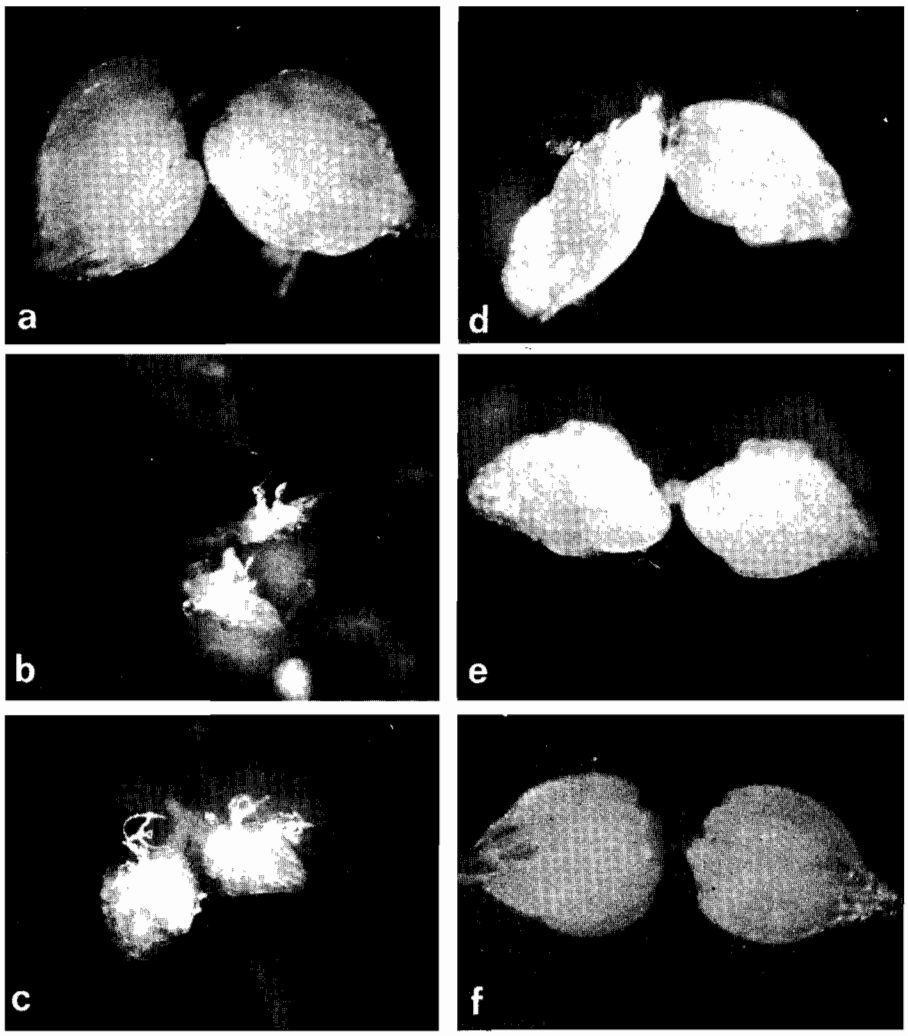

Fig. 1. Ovaries of *Drosophila melanogaster*, removed from females and photographed in saline. ×30. All animals were treated with 0.2 µl acetone, sometimes containing 4 µg precocene-2 (CalBiochem) and/or 0.5 µg juvenile hormone-2 (JH-2) (CalBiochem), which was applied to the abdomen of a female 0–4 h after adult ecdysis. *a* 48-h *Ore-R-C*, acetone control; *b* 48-h *Ore-R-C*, precocene; *c* 48-h *ap*^{4}/*ap*^{4}, acetone control; *d* 48-h *Ore-R-C*, precocene and JH-2; *e* 48-h *ap*^{4}/*ap*^{4}, JH-2; *f* 144-h *Ore-R-C*, precocene.

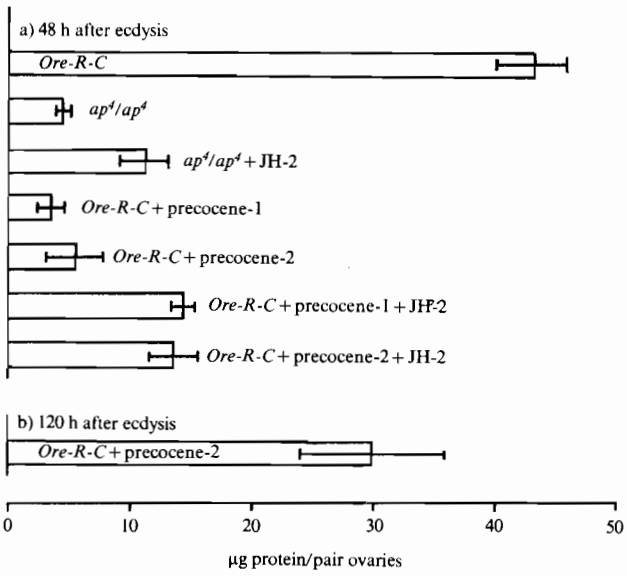

Fig. 2. Protein content of *Drosophila melanogaster* ovaries. Lowry test[13] with bovine serum albumin standard. 0.2 µl acetone, sometimes containing 4 µg precocene-1 or -2 (CalBiochem) and/or 0.5 µg JH-2, was applied to the abdomen of a female 0–4 h after adult ecdysis. Samples were taken 48 or 120 h after treatment. Mean protein data based on at least 5 determinations on 10 pooled ovaries. Error bars indicate 2 SD.

(figure 1, d). Quantitatively similar rescue occurred when juvenile hormone was administered to ap^4 homozygotes (figure 1, e). Thus, the major effects of precocene on vitellogenesis apparently stem from interference with the normal functioning of the juvenile hormone system.

Total ovarian protein was measured in all of the experimental groups. This quantitative data on protein content (figure 2, a) supports the conclusions already drawn from inspection of ovaries (figure 1).

Several laboratories have reported that precocene-treatment produces abnormal morphology or function of the corpora allata in *Oncopeltis*[8,9], *Locusta*[10,11] and *Periplaneta*[12]. Ultrastructural evidence of massive autophagy and cellular degradation or collapse suggested that the chemical allatectomy is irreversible[9,11]. Our results with *Drosophila* differ; the flies can significantly recover from precocene effects. *Ore-R-C* females, treated with precocene at ecdysis, show very little vitellogenesis at 2 days (figures 1, b and 2, a) but significant vitellogenesis at 6 days (figures 1, f and 2, b). In addition, the fact that precocene-treated flies do not show the premature death characteristic of adult ap^4 homozygotes argues for reversibility of the precocene effects.

Our demonstration that precocene inhibits vitellogenesis in *Drosophila* allows many new experimental approaches to studies of vitellogenesis and juvenile hormone action in a defined genetic context. In addition, our demonstration that precocene inhibition is reversible suggests that the hypothesis of precocene action by its selective cytotoxic attack on the corpora allata deserves further critical investigation, at least for *Drosophila*.

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2 W.S. Bowers, T. Ohta, J. Cleene and D. Marsella, Science *193*, 542 (1976).
3 M. Pener and L. Orshan, Israel J. Zool. *26*, 262 (1977).
4 J.H. Postlethwait and K. Weiser, Nature New Biol. *244*, 284 (1973).
5 J. Gavin and J. Williamson, J. Insect Physiol. *22*, 1457 (1976).
6 M. Kambysellis, Am. Zool. *17*, 535 (1977).
7 A. Handler and J.H. Postlethwait, J. exp. Zool. *206*, 247 (1978).
8 W.S. Bowers and R. Martinez-Pardo, Science *197*, 1369 (1977).
9 G. Unnithan, K. Nair and W.S. Bowers, J. Insect Physiol. *23*, 1081 (1977).
10 M. Pener, L. Orshan and J. DeWilde, Nature *265*, 548 (1977).
11 H. Schooneveld, Experientia *35*, 363 (1979).
12 G. Pratt and W.S. Bowers, Nature *265*, 548 (1977).
13 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).

Intracellular distribution of estrogen receptors: A function of preparation[1]

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Summary. The intracellular distribution of unbound estrogen receptor was estimated using nonaqueous and aqueous isolation of nuclei and autoradiography. The estimated amount of nuclear receptor varied greatly with the procedure used and thus caution is urged in approximating the in vivo intracellular distribution of receptors.

The most widely accepted model for the mechanism of action of steroids was developed from a plethora of studies concerning the uptake and retention of estradiol by the female reproductive tract. According to this model, estradiol enters the uterine cells, binds to a specific high-affinity cytoplasmic receptor and then is translocated to the nucleus by a temperature-sensitive process[2,3]. However, there are data in the literature which question this model[4] and which suggest that the amount of unbound receptor found in a given preparation might be more a function of the isolation procedure rather than a true representation of the in vivo intracellular distribution of receptors[5–8]. In the experiments described below, different methods used to isolate nuclei were compared in 2 different tumor systems.

Methods. Cells from a breast tumor line, designated MCF-7 (kindly supplied by Dr W. McGuire, Department of Medicine, The University of Texas Health Science Center at San Antonio) and cells obtained from the pleural cavity of a patient with advanced breast cancer were used in these experiments. Labeled estradiol was purchased from Amersham/Searle (106 Ci/mM) and unlabeled estradiol was purchased from Sigma.

Standard preparation of nuclei: Cells were incubated in MEM (Grant Island Biological Co.) for various periods of time in the presence of various concentrations of steroids [^{3}H-estradiol ($1-5\times10^{-9}$ M) or ^{3}H-estradiol ($1-5\times10^{-9}$ M) + unlabeled estradiol ($1-5\times10^{-7}$M)]. The cells were then washed in TRIS buffer (50 mM TRIS, 5 mM Mg,