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Ecdysteroid Production and Metabolism by an Epithelial Cell Line from *Chironomus tentans*

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Insect cell lines have been shown to be useful systems for the study of ecdysteroid action. They have been used primarily to demonstrate intracellular ecdysteroid receptors and to study the regulation of ecdysteroid-dependent differentiation [1]. In these experiments it is essential to know whether the cells produce ecdysteroids themselves and how stable exogenously applied ecdysteroids are.

Only recently insect cell lines have been studied under the aspect of ecdysteroid production [2]. Among several lepidopteran lines, only those of *Trichoplusia ni* and *Manduca sexta* and a line from the cockroach *Blattella germanica* were able to produce ecdysteroids. Interestingly, the ecdysteroid-producing lines were able to synthesize chitin without further hormonal stimulation. The hypothesis was put forward whether the priming effect brought about by ecdysteroids to initiate chitin synthesis is unnecessary in these cell lines due to their endogenous production of ecdysteroids. Since the *Chironomus* cell line synthesizes chitin in considerable amounts without hormonal stimulation [3], it was worthwhile to test this hypothesis.

With a radioimmunoassay (RIA), using antiserum ICT-I [4] and an extraction and purification procedure for ecdysteroids as described in [5], we looked for RIA-positive material in the cell culture medium before exposure to cells, in cells, and in the medium after cells had been grown in it for 1 to 3 weeks. There was no detectable RIA-positive material in the cells, whereas both fresh medium and medium in which cells had been grown contained RIA-positive material. The concentrations were between 30 and 50 pg ecdysone equivalents/ml for the medium and increased after cells had been grown for 1 week up to tenfold (2×10^{-9} *M* as the maximal value). If cells were cultured for longer periods, the concentration of RIA-positive material decreased. The presence of about the same amount of RIA-positive material in fresh culture medium without cells grown in it has been shown in two other investigations [2]. They also noted in a cockroach line that after about 2 weeks the amount of secreted RIA-positive material decreased.

The extracted and partially purified material was separated on reversed-phase HPLC. All RIA-positive material in the medium elutes in the highly polar fraction, whereas medium from cultured cells, in addition, contained material cochromatographing with authentic ecdysone (Fig. 1). The cell line from the cockroach *Blattella germanica* also secreted only ecdysone, whereas the titer of 20-OH-ecdysone increased with time in the lepidopteran cell line developed from *Trichoplusia ni* [2].

The *Chironomus* cells are able to convert both ^{3}H-ecdysone and ^{3}H-ponasterone A into highly polar products. Ecdysone is also metabolized to less polar substances. After 3 days of incubation with ^{3}H-ecdysone 45 % of the radioactive material chromatographs in the ecdysone region, 34 % is in the apolar region, and 20 % in the polar one. After 5 days of incubation only 10 % of ecdysone is left, 10 % is in the apolar fractions, and 80 % in the polar ones. The cells are not able to convert ecdysone to 20-OH-ecdysone, even after longer periods of incubation. Exogenously applied 20-OH-ecdysone in concentrations between 10^{-8} and 10^{-6} *M* remains quite stable during 1 week of incubation. Only 25 % of the hormone is lost during this time (Table 1). This means that long-term incubations with 20-OH-ecdysone, which are necessary, e.g., for the "induction" of acetylcholinesterase [6] or the inhibition of chitin synthesis [3], can be performed without the multiple addition of hormone.

The *Chironomus* cell line is of embryonic origin but it has clearly epidermal or imaginal disk characteristics, e.g., the formation of epithelial vesicles [6, 7], the synthesis of chitin [3], the presence of dopadecarboxylase, and the hormonal regulation of these processes [1, 3]. The synthesis of ecdysteroids by the epithelial cell line from

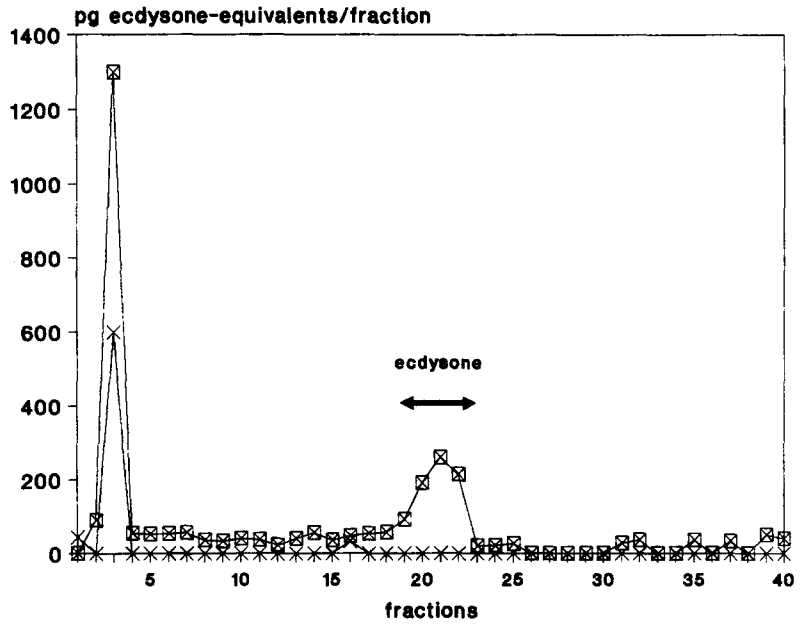

Fig. 1. Chromatography of secreted ecdysteroids from the medium of the *Chironomus tentans* cell line (⊠). Ecdysteroids were purified [5] and injected on a reverse-phase column, μBondapak C_{18} (particle size 10 μm; i.d. 7.8 mm × 30 cm; Waters Ass.) and eluted with methanol-water (4:6; v/v). The flow was kept constant at 1.5 ml/min and 200 drops per fraction were collected. Beginning with fraction 25 the solvent was changed to 100% methanol. Each fraction was dried and used for RIA with the antibody ICT-I [4]. Authentic 20-OH-ecdysone and ecdysone elute in the fractions 9–11 and 19–23, respectively. (×) represents the values from fresh, unused culture medium, which was treated in the same way

Table 1. Metabolism of exogenously applied 20-OH-ecdysone by the epithelial cell line from *Chironomus tentans*. After the cells were grown for 10 days 20-OH-ecdysone was added. Immediately after mixing (day 0) and 3 and 7 days later, medium was collected, and ecdysteroids were extracted and separated by reversed-phase HPLC; RIA activity was determined in four pooled samples from each run. For comparison, fresh medium was treated in the same way; no RIA activity in the three fractions (20-OH-ecdysone, ecdysone, and apolar) was detected in this control (means ± SD, $n = 3$)

	Ecdysteroids [ng/ml]		
Days of incubation with hormone	20-OH-Ecdysone	Ecdysone	Apolar
0	189.7 ± 29.5	1.0 ± 0.06	0.1 ± 0.04
3	138.2 ± 27.0	1.2 ± 0.10	0.2 ± 0.06
7	146.2 ± 17.7	1.1 ± 0.20	0.2 ± 0.03

Chironomus tentans is in accordance with reports that insect epidermis is able to synthesize ecdysteroids, as summarized recently [8], but it is the first demonstration of ecdysteroid production in a dipteran cell line. The low levels of ecdysone synthesized by the cell line from *Ch. tentans* do not effect vesicle formation, growth, and differentiation in the cell line. This is in contrast to the lepidopteran cell line from *Trichoplusia ni,* which also grows in multicellular vesicles but changes to the formation of aggregates, when it attains the capacity to produce ecdysteroids [9] in similar concentrations [2].

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