EFFECT OF JUVENILE HORMONE ON CIRRIPED METAMORPHOSIS

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SUMMARY

The synthetic insect growth regulator, ZR-512 (2), causes precocious metamorphosis of the cyprid larva of the acorn barnacle, Balanus galeatus. Juvenile hormone (JH) (1) was also found to be effective in this assay at a threshold concentration of 10^3 ppb, three orders of magnitude less active than ZR-512. Pretreatment of cyprids with certain subthreshold concentrations of JH partially inhibited the expected metamorphic activity of subsequently added ZR-512, suggesting that the two compounds interact with the same receptor.

Considering the extensive literature devoted to the directive role played by the juvenile hormone (JH) (1) in insect metamorphosis, studies of a presumably comparable system in the Crustacea are of particular interest. Experimental application of JH or a synthetic analogue such as ZR-512 (2) (Henrick et al 1973) will alter the development of the holometabolous insect to the extent of producing defective pupal-adult intermediates (Gilbert and Schneiderman 1960; Schneiderman 1971) or may prevent metamorphosis altogether (Wigglesworth 1970). This laboratory has previously demonstrated that ZR-512 exerts a dramatic effect upon the cyprid stage of the acorn barnacle, Balanus galeatus (Gomez et al 1973). When subjected to ZR-512, laboratory-reared cyprids will undergo precocious metamorphosis to the adult morph without attaching to a substrate. Attachment normally precedes metamorphosis, where it is a necessary condition for proper positioning on a substrate and establishment of an effective feeding

posture. Thus, the hormone applied at specific times during development proves fatal to both the insects and this Crustacean but in the former metamorphosis is impeded while in the latter it is prematurely induced. This correlation has instigated further research into the chemical mechanisms mediating Crustacean metamorphosis,and the effects of synthetic juvenile hormone, both alone and in conjunction with ZR-512, upon barnacle cyprid metamorphosis are reported here.

The juvenile hormone was freshly synthesized by an established route (Faulkner and Petersen 1973). The sample consisted of a 1:1 racemic mixture of both diastereoisomeric epoxides. This preparation had been shown to be highly active when tested on Tenebrio molitor (J. D. O'Connor, personal communication). The JH preparation was dissolved in acetone to make a stock solution (15 mg/ml) which was stored at -20°C for short periods. Aliquots were diluted with acetone such that addition of 0.5 ml portions of the acetone solutions to 200 ml of filtered seawater yielded final JH concentrations from 0.01 to 5 ppm. Acetone (0.5 ml) was added to filtered seawater (200 ml) in the control experiments.

Adult Balanus galeatus, collected by SCUBA from nearshore waters off La Jolla, released Stage I nauplii which were subsequently reared to the cyprid stage in the laboratory by the established methods (Mollenock and Gomez 1972). Cyprid larvae were maintained at 25°C $\pm$ 2° in 250 ml beakers containing filtered seawater and various concentrations of JH for 6 days, at which time the numbers of metamorphosed adults were recorded (Table 1). In the second series of experiments, the newly emerged cyprids were initially maintained in beakers containing the various concentrations of JH in 200 ml filtered seawater. After 2 days, the cyprids were pipetted into new beakers

TABLE 1

Results of 6 Days Exposure to Juvenile Hormone on Balanus galeatus Cyprids

JH (ppb)	Number of Cyprids M	U	D	% MET.	N
Control (Acetone)	0	61	12	0	3
10^{-1}	0	55	6	0	3
5×10^{-1}	0	70	17	0	3
1	0	54	14	0	4
10	0	88	7	0	4
5 x 10	0	74	15	0	3
10^2	0	79	11	0	4
5×10^2	0	82	17	0	4
10^3	3	89	24	3	5
5×10^3	63	32	18	66	5

TABLE 2

Results of 2 Days Exposure to Juvenile Hormone Followed by 6 Days Exposure to ZR-512 (10 ppb) on Balanus galeatus Cyprids

JH (ppb)	Number of Cyprids M	U	D	% MET.	N
Control (Acetone-Acetone)	0	55	8	0	2
Control (Acetone-ZR-512)	69	5	10	93	2
10^{-2}	65	5	7	92	4
10^{-1}	72	8	11	90	4
5×10^{-1}	58	12	14	83	4
1	47	26	9	64	5
10	23	86	15	21	5
5 x 10	25	71	12	26	5
10^2	33	74	18	31	6
5×10^2	41	49	10	46	4
10^3	45	58	14	44	5
5×10^3	75	33	17	69	5

Tables 1 and 2

The mean values are recorded:

M, metamorphosed
U, unmetamorphosed
D, death (recorded at termination of each experiment)
% MET, percentage metamorphosis
N, number of replicative experiments

Table 1 - Total number of cyprids = 750

Table 2 - Total number of cyprids = 1296

containing ZR-512 (10 ppb) in 200 ml filtered seawater. Numbers of metamorphosed adults were recorded after 6 days of chemical exposure (Table 2).

Comparable to the ZR-512 results (Gomez et al 1973), JH caused precocious metamorphosis in Balanus galeatus cyprids (Figure 1). The threshold concentration for JH was found to be

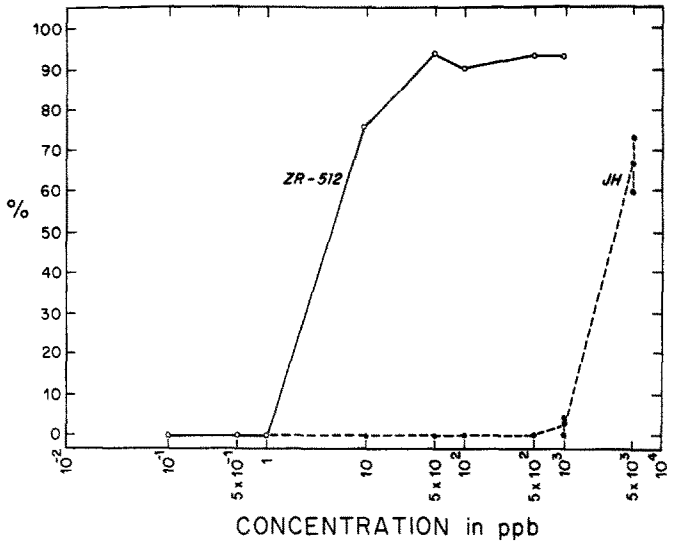
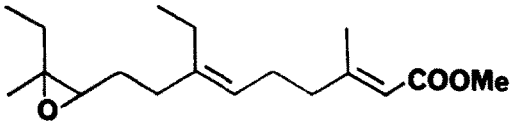


Figure 1. The mean percentage of cyprid metamorphosis after 6 days as a function of hormone concentration.

10^3 ppb; thus, JH was three orders of magnitude less effective than ZR-512. This result was not unexpected since Henrick et al (1973) had found that both ZR-512 (2) and ZR-515 (3) are more effective in certain insect bioassays than the natural Cecropia (1) JH. Since our sample of JH is a 1:1 mixture of two racemic diasteroisomers, one could expect that a natural Cecropia JH sample would be slightly more active but no greater than one order of magnitude.

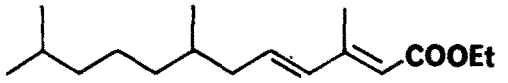
In the second series of experiments, it was found that the effectiveness of ZR-512 could be altered by first pretreating the

(1)



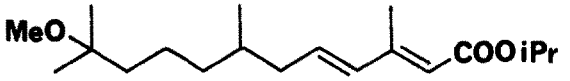
Juvenile Hormone: (methyl 10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate)

(2)



ZR-512: (3,7,11-trimethyldodeca-2,4-dienoate)

(3)



ZR-515: (isopropyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate)

cyprid cultures with various concentrations of JH, followed by applications of ZR-512. Very low concentrations of JH (less than 0.5 ppb) had no effect upon the cyprids, so that subsequent addition of ZR-512 gave similar results to those obtained with a single treatment of 10 ppb ZR-512 (Figures 1 and 2). The highest concentration of JH (5×10^3 ppb) produced results comparable to those of tests in which only 5×10^3 JH were employed. Pretreatment of cyprids with JH at concentrations between 0.5 and 10 ppb, followed by the addition of ZR-512 (Figure 2), resulted in an inhibition of the expected metamorphic activity of the ZR-512

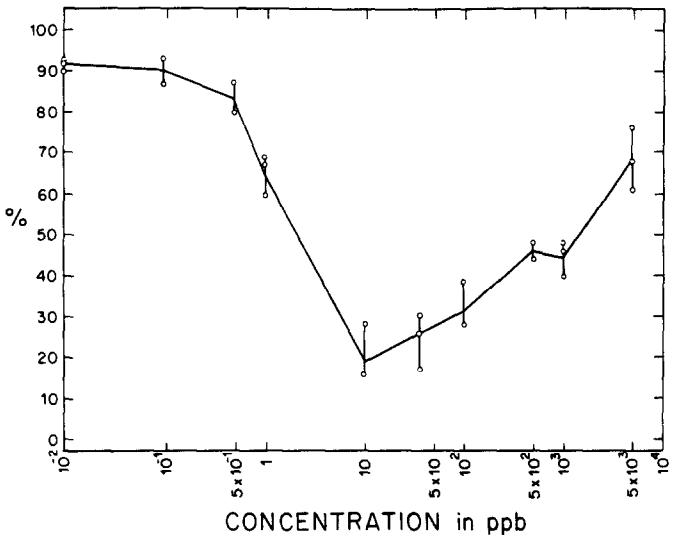


Figure 2. The mean percentage and range of variations with replicative experiments of cyprid metamorphosis after 2 days pretreatment of JH and subsequent exposure to ZR-512 (10 ppb) for 6 additional days, as a function of concentration of JH.

(Figure 1). Maximum inhibition occurred when the concentrations of JH and ZR-512 were approximately equal at 10 ppb (Figure 2). The inhibitory influence of JH diminished as the concentration of the JH approached threshold, between 10 ppb and 10^3 ppb.

These experiments illustrate that JH, when applied alone at concentrations of 10^3 ppb and above, induces precocious metamorphosis in Balanus galeatus cyprids. Yet, when tested in conjunction with ZR-512, JH serves to alter the expected metamorphic activity of ZR-512. This suggests that the inhibition of the ZR-512 activity is caused by blockage of the receptor site by the less active agent, JH.

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