Bull. Inst. Zool., Academia Sinica **28**(4): 275-280 (1989)

EFFECT OF INSECT HORMONES ON INFECTION OF NUCLEAR POLYHEDROSIS VIRUS TO PRIMARY CULTURED CELLS OF *BOMBYX MORI* L.

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(Accepted June 5, 1989)

Shin-Yuan Su, Feng-Kuo Hsieh and Roger F. Hou (1988) Effect of insect hormones on infection of nuclear polyhedrosis virus to primary cultured cells of *Bombyx mori* L. *Bull. Inst. Zool., Academia Sinica* **28**(4): 275-280. Susceptibility of ovarian cells and hemocytes of the silkworm, *Bombyx mori* L., to nuclear polyhedrosis virus (BmNPV) was studied using primary cultures. Juvenile hormones (JH) II and III at 30 or 75 μg/ml, and JH-I at 75 μg/ml in the cultured media of ovarian cells inhibited BmNPV infection. The presence of JH-I, II, III at 75 μg/ml also inhibited BmNPV infection in hemocytes cultures. Beta-ecdysone at 30 μg/ml in the cultured media of ovarian cells inhibited BmNPV infection, but enhanced the viral infection in hemocytes at 15 or 30 μg/ml.

Key words: *Bombyx mori*, BmNPV, Juvenile hormone, β-ecdysone, Cell culture.

Injection of an ecdysone analogue into debrained dauerpupae of the silkworm, *Bombyx mori* L., resulted in an enhanced susceptibility to nuclear polyhedrosis virus (NPV) infection (Watanabe and Aruga, 1970). Studies with the isolated larval abdomens of the silkworm gave similar results (Kobayashi and Yamaguchi, 1970, 1972). In addition, Keeley and Vinson (1975) pointed out that replication of NPV in *Heliothis virescens* was reduced after injecting with β-ecdysone. Liu and Hou (1985) investigated the effect of a cocooning regulator containing ecdysterone on infectivity of NPV in the silkworm, and proposed that ecdysteroids might be capable of suppressing the development of nucleopolyhedrosis in *B. mori* when treated before NPV inoculation. Therefore, it is conceivable that NPV infection is related to hormonal action.

Progress in culturing insect tissues and cells during the last decade has enable us to use in vitro systems for studying the action of hormones at morphological, physiological and biochemical levels. But most attention has been focused on the mechanisms of insect molting and juvenile hormones (JH) action on target cells in insect tissues culture systems. Kloc *et al.* (1984) indicated that JH-III (87.5 μg/ml) or β-ecdysone (17.5 μg/ml) in the culture medium of *Estigmene acrea* cells inhibits the viral replication cycle. This infectious cycle returns to normal upon removal of the hormones from the cell culture medium. In the present study, we investigated

effect of JH-I, II, III and β-ecdysone on infection of *B. mori* NPV (BmNPV) to the primary cultured cells.

MATERIALS AND METHODS

A drop of hemolymph from the 5th instar silkworm was added to the Grace's medium. Most of the hemocytes sank and attached themselves to the bottom within a few minutes. After washing with Grace's medium, 10% fetal bovine serum was added to the Grace's medium containing penicillin and streptomycin. In addition, the 2-day-old female pupae were surface-sterlized prior to the dissection. Their ovarioles were pulled out with fine forceps and needle under a stereomicroscope, and then were torn into small pieces. The ovarian cells were washed by changing the Grace's medium several times after settling. Then, silkworm haemolymph of the 4-day-old 5th instar was added to the culture medium to make up 10% of final concentration.

The β-ecdysone (Sigma Chemical Co., St. Louis, Mo. USA) was dissolved in the Grace's medium salt solution, sterilized by filtrating through a 0.22 μm filter and frozen until use. JH-I, II and III (Sigma Chemical Co., St. Louis, Mo. USA) were dissolved in dimethyl sulfoxide (DMSO). The hormones were added to the media at the beginning of culturning, and the primary cultured cells were inoculated with culture medium supernatants containing infectious BmNPV at 24 hr after hormonal treatment. The $TCID_{50}$ titre of the BmNPV inoculum, using Bm-N as the indicator cell line, was 3.8×10^5/ml. The inoculated cultures were incubated at 27±1°C for 96 hr to allow polyhedral formation. Estimation of the percentage of BmNPV infection was carried out by placing the culture vessel on the stage of a phase contrast inverted microscope and 10 fields on the bottom of the vessel were counted.

RESULTS AND DISCUSSION

The effect of insect hormones on infection of BmNPV to the primary cultured ovarian cells is shown in Table 1. When the concentrations of JH-II and JH-III were 30 or 75 μg/l in the medium, the percentage of infected cells was low. It indicated that JH-II and JH-III in the medium could inhibit virus infection to the primary cultured ovarian cells, but their inhibitory effect was better at 30 μg/ml than at 75 μg/ml. Inhibition of JH-I to susceptibility of the cultured ovarian cells to BmNPV infection was effective at 75 μg/ml but not at 30 μg/ml. Beta-ecdysone inhibited BmNPV infection to cultured ovarian cells by adding 30 μg/ml to the medium, while a lower concentration at 15 μg/ml was not inhibitory. Table 1 shows that those treatments with higher percentage of infected cells have more number of polyhedra per infected cell. In particular, treatments with β-ecdysone or JH-II have more number of polyhedra per infected cell than others.

Table 2 shows the effect of insect hormones on BmNPV infection to the primary cultured hemocytes of *B. mori*. The presence of JH-I, II and III at 75 μg/ml in the cultured medium of hemocytes had an inhibitory effect on BmNPV infection. However, the susceptibility of the cultured hemocytes to BmNPV infection was enhanced by β-ecdysone at both 15 and 30 μg/ml.

The presence of JH in the culture media might inhibit nuclear DNA, RNA and protein syntheses, and cell propagation (Cohen and Gilbert, 1972; Lezzi and Wyss, 1976; Himeno *et al.*, 1979; Kloc *et al.*, 1984). Therefore, as suggested by Kloc *et al.* (1984), it is possible that inhibition of NPV replication in the cultured ovarian cells in the presence of JH is due to the hormonal block of host DNA synthesis, the shutdown of host

Table 1
Effect of β-ecdysone and juvenile hormones on cultured ovarian cells of *B. mori* inoculated with BmNPV(1)

Treatment(2)	Conc. (μg/ml)	Cells with PIBs(3) (%)	Number of PIBs/Infected cell
β-ecdysone	15	74.69 *cd*	9.97
	30	56.86 *a*	8.32
JH-I	30	81.83 *d*	3.52
	75	57.62 *a*	3.38
JH-II	30	53.00 *a*	2.43
	75	67.07 *b*	3.70
JH-III	30	53.39 *a*	6.34
	75	70.40 *bc*	8.17
Medium only	0	74.04 *bcd*	3.41
DMSO (μl/ml)	12.5	77.84 *d*	—

(1) Ovarian tissues for the primary cultures were obtained from 2-day-old female pupae.
(2) Beta-ecdysone was dissolved in the Grace's medium, and juvenile hormone I, II and III were dissolved in dimethyl sulfoxide (DMSO) and then added to media giving a final concentration of DMSO less than 12.5 μl/ml.
(3) Means followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

Table 2
Effect of β-ecdysone and juvenile hormone on *B. mori* primary cultured hemocytes inoculated with BmNPV(1)

Treatment(2)	Conc. (μg/ml)	Cells with PIBs (%)(3)	
		V0	V7
β-ecdysone	15	80.45 *b*	7.79 *e*
	30	93.01 *b*	11.16 *f*
JH-I	30	—	6.43 *de*
	75	—	2.51 *ab*
JH-II	30	—	4.15 *bcd*
	75	—	0.61 *a*
JH-III	30	—	4.48 *bcd*
	75	—	3.31 *bc*
Medium only	0	30.96 *a*	5.51 *cd*
DMSO (μl/ml)	12.5	—	4.06 *bcd*

(1) Hemocytes for the primary cultures were obtained from the newly-molted and 7-day-old female 5th instar (V0 and V7).
(2) Beta-ecdysone was dissolved in the Grace's medium, and juvenile hormone I, II and III were dissolved in dimethyl sulfoxide (DMSO) and then added to media giving a final concentration of DMSO less than 12.5 μl/ml.
(3) Means followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

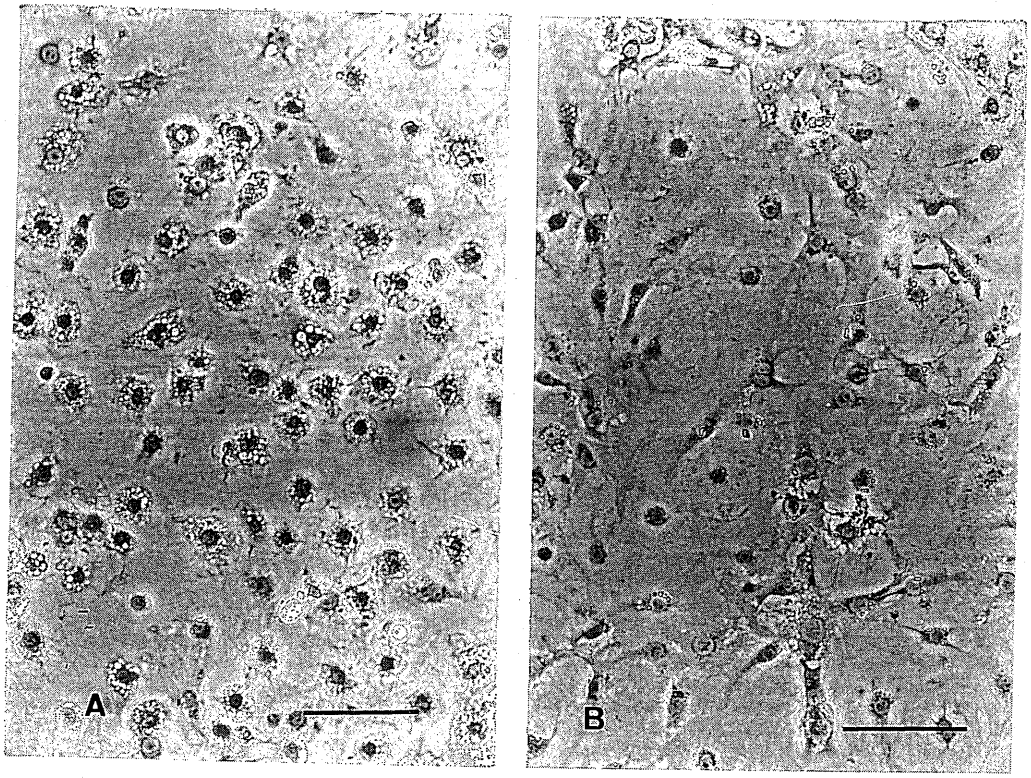

Fig. 1

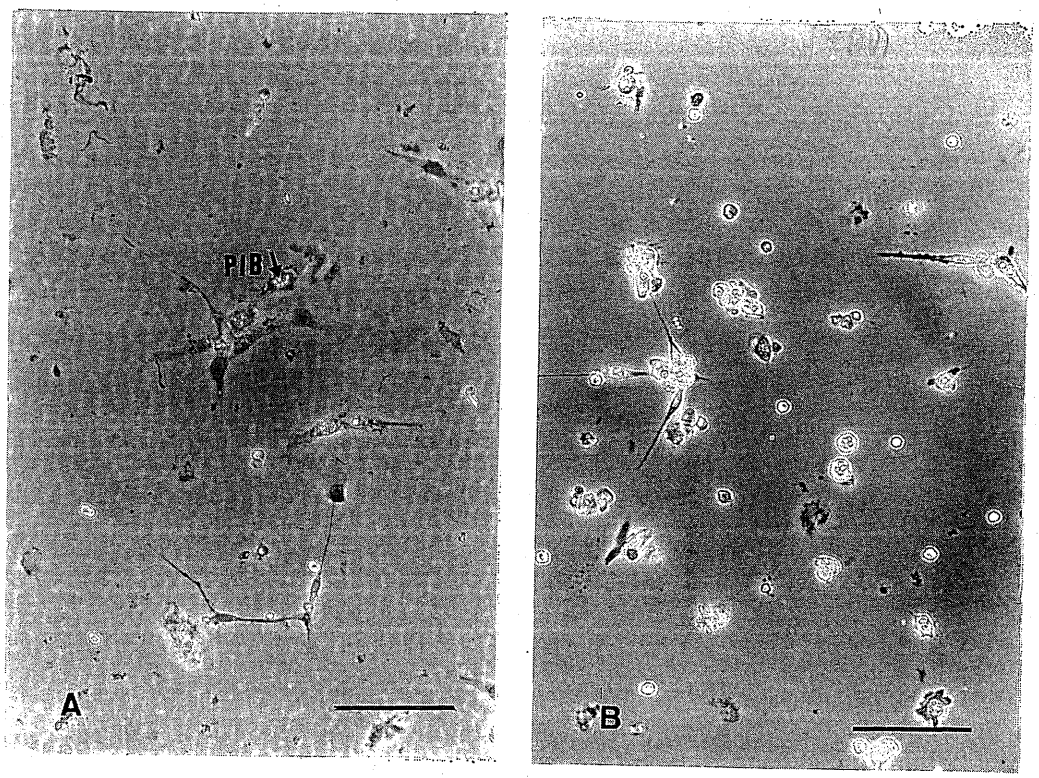

Fig. 2

RNA and/or protein synthesis. However, Volkman *et al.* (1987) have found that cytochalasins B and D prevent the production of infectious budded *Autographa californica* NPV by inhibiting synthesis of complete virions. In the presence of cytochalasins B and D, particles lacking nucleocapsids bud from the plasma membrane of AcNPV infected cells, implicate microfilaments to be involved in the synthesis, transport and/or assembly of critical virus components. Furthermore, we cannot neglet the possibility that JH can act through the modification of the microfilaments since alteration of vacuolated cytoplasm was observed on the JH-treated ovarian cells (Fig. 1A, B).

It has been shown that activated metabolism plays an active role in NPV development, possibly due to the increased rate of synthesis of viral constituents (Stairs, 1970; Kobayashi and Kawase, 1980). Working with a clonal subline of the Kc cells, Wyss (1976) demonstrated a slight increase in cell proliferation with low concentrations of ecdysone (6.3×10^{-7} M) and 20-hydroxyecdysone (6.3×10^{-9} M) in *Drosophila*. At high concentrations, ecdysteroids inhibited cell proliferation. Similar results were obtained from other cultured cells (Mitsuhashi and Grace, 1970; Courgeon, 1972). This may explain why β-ecdysone with a high concentration (30 μg/ml) inhibits BmNPV infection in the culture medium of ovarian cells.

The primary cultured hemocytes exposed to ecdysteroids seemed to be less adherent to plastic surface of the culture vessel. There is an alteration of cell morphology from the normal shape with flattening and spreading to spherical shape (Fig. 2A, B). Similarly, Judy (1969) indicated that both larval and pupal hemocytes responded to ecdysterone treatment by increasing membrane activity and cell movement, whereas injection with solvent did not show such a response. Therefore, it is speculated that enhancement of BmNPV infection in the primary cultured hemocytes in the presence of β-ecdysons is due to increase in membrane activity by hormones.

From these results, it is visualized that the effects of insect hormones on the susceptibility of cultured cells to virus infection are affected by concentrations of hormones and the tissues that cells derived from.

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Fig. 1. Ovarian cells of *Bombyx mori* in primary culture for 4 days. A, cells without JH treatment showing vacuolated cytoplasm; B, cells treated with JH-III at 75 μg/ml showing clear cytoplasm. Bar=100 μm.

Fig. 2. Primary culture of Hemocytes infected with BmNPV 4 days post inoculation. A, cells without β-ecdysone treatment showing normal shape, PIB: polyhedral inclusion boby; B, cells treated with β-ecdysone at 30 μg/ml showing spherical shape. Bar=100 μm.

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昆蟲激素對核多角體病毒感染家蠶初代培養細胞之影響

蘇 新 元　謝 豐 國　侯 豐 男

將昆蟲激素分別添入家蠶卵巢及血球初代細胞培養液中，培養細胞之形態及其對 BmNPV 之感受性皆受到影響。青春激素 JH-I、JH-II 和 JH-III 75 μg/ml 添入培養液中，降低卵巢及血球培養細胞對 BmNPV 之感受性；JH-II 和 JH-III 30 μg/ml 仍可抑制 BmNPV 感染卵巢培養細胞。β-脫皮素 30 μg/ml 對不同種類培養細胞具不同的影響，其降低卵巢培養細胞對 BmNPV 之感受性，却提高血球培養細胞對 BmNPV 之感受性。