

RELATIONSHIP BETWEEN ECDYSTEROIDS AND $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ ACTIVITY OF OVARIAN MICROSOMAL FRACTION IN *HAEMAPHYSALIS* *LONGICORNIS* NEUMANN*

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Abstract In order to explore the role of ecdysteroids in reproduction of ixodid ticks, the relationship between ecdysteroids levels and $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity of *Haemaphysalis longicornis* ovarian microsomal fraction is studied. The results indicate that before oviposition the quantitative variation of both ecdysteroids and $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activities is not significant. at oviposition day, the enzyme activity is highest. The level of endogenous ecdysteroids is coincident with its enzyme activity. Exogenous ecdysteroids stimulate its enzyme activity, but it shows no dose-dependent relationship.

Keywords *Haemaphysalis longicornis* Neumann; reproduction; $\text{Na}^+ - \text{K}^+ - \text{ATPase}$; ovarian microsomal fractions

Ecdysteroids regulate the moulting process and reproduction in insects. These hormones also occur in ticks, but less is know about them in reproduction. Jiang et al^[1] reported that ecdysone (E) and 20-hydroxy ecdysone (20-E) existed in adult females of *Dermacentor niveus* and *Haemaphysalis longicornis*, but how is influential on reproduction and whether the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity has relationship with reproduction don't yet know. This paper is to study the effects of both endogenous and exogenous ecdysteroids on $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity and their relationship.

1 Materials and methods

1.1 Ticks The females used in this study were taken from a colony *H. longicornis* which was collected in Beijing and reared in the laboratory at $(25 \pm 1)^\circ\text{C}$, 75% relative humidity in incubators plugged with absorbent cotton with illumination of 6 hours per day.

1.2 Extraction of ecdysteroids The methods was according to the previously described^[1].

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1.3 High performance liquid chromatograph (HPLC) The HPLC apparatus was model LC-4A (Schmadzu Inc.) equipped with a 254 nm UV-detector. The column was a 5 μ m ZORBAX ODS, 4.6 mm \times 150 mm. The solvent system was methanol-water solution ($V:V=1:1$), the flow rate was 1.0 mL \cdot min $^{-1}$ and column temperature was 30 $^{\circ}$ C.

1.4 Preparation of ovarian microsome The ovaries were removed under ice-cold saline and stored in the saline on ice, then homogenized in extraction buffer (0.05 mol \cdot L $^{-1}$ Tris-HCl pH8.5, 0.25 mol \cdot L $^{-1}$ NaCl, 1 mmol \cdot L $^{-1}$ PMSF, 5 mmol \cdot L $^{-1}$ NaHSO $_3$) with desirable volume. Homogenates were centrifuged at about 600 g for 15 min. The aqueous layer was removed and stored, the precipitate was suspended in original volume extraction buffer and centrifuged again and removed the aqueous layer, the combination aqueous layer were centrifuged at about 1.35 \times 10 4 g for 30 min. The supernatant was removed and centrifuged at 1.05 \times 10 5 g for 60 min, discarded the supernatant, dissolved the sediment with 1 mL extraction buffer and obtained ovarian microsomal fraction. These operation were carried out at 0~4 $^{\circ}$ C.

1.5 Na $^{+}$ -K $^{+}$ -ATPase activity assay Enzymic activity determination was based on Ilenchuk and Davey^[2]. The phosphate released in media was measured by the colorimetry of Fiske and Subbarow^[3] and the protein content determined using a commercially available kit (Bio-Rad) based on the techniques of Bradford^[4]. The Na $^{+}$ -K $^{+}$ -ATPase activity was expressed as mmol \cdot g $^{-1}$ \cdot h $^{-1}$ (Pi(inorganic phosphate) released protein).

1.6 Hormone treatment 20-E was purchased from Aldrich Chem. Co. and diluted in appropriate ethanol to 10, 20, 40 g \cdot L $^{-1}$ and applied topically 1 μ L to the female *H. longicornis* at 3rd day after engorgement respectively, after two days, removed the ovaries and prepared the ovarian microsomal fractions and assayed the enzyme activities.

2 Results

2.1 Ecdysteroid titers in the haemolymph and ovary The ecdysteroid titers in the hemolymph of *H. longicornis* was very low before engorgement(37.5 mg \cdot L $^{-1}$) then increased and it was peaked prior to oviposition (153.1 mg \cdot L $^{-1}$), and decreased to 48.1 mg \cdot L $^{-1}$ at 3rd day after oviposition (Tab. 1).

Before engorgement, there was little of ecdysteroids level in the ovary, then rose rapidly from the 3rd day after engorgement (270 ng/tick) to oviposition (770 ng/tick)(Tab.1).

Table 1 Ecdysteroid titers in *H. longicornis* haemolymph and ovary at different physiology periods

Physiology periods	ecdysteroid titers	
	(M(haemolymph) \pm s)/(mg \cdot L $^{-1}$)	(M(ovary) \pm s)/(ng/tick)
Before engorgement day	37.5 \pm 2.4	62.0 \pm 5.6
Engorgement day	60.0 \pm 4.3	82.0 \pm 6.3
3rd day after engorgement	85.0 \pm 4.0	270.0 \pm 12.4
Oviposition day	153.1 \pm 10.4	770.0 \pm 35.6
3rd day after oviposition	48.1 \pm 3.8	380.0 \pm 24.5

2.2 Enzymic activities of ovarian microsomal fraction The $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity showed little amount before 3rd day after engorgement then rose sharply to $7.06 \text{ mmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ (Pi to protein) at oviposition day, then decreased to a low level (Tab.2).

2.3 Effect of exogenous 20-E on enzymic activity of the 3rd day after engorgement ovarian microsomal fraction At the 3rd day after engorgement, ticks were applied topically with different dosage of 20-E, then after two days, determined the enzyme activity. The results indicated that 20-E stimulated enzyme activity, but showed no dose-dependent relationship within some range. At the dose of 10, 20, 40 $\mu\text{g}/\text{tick}$, the activity was 1.70, 1.78, 1.75 $\text{mmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ respectively (Tab. 3).

Table 2 $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activities of *H. longicornis* ovarian microsomal fraction at different physiology periods

Physiology periods	$\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activities ($\text{mmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$)
Engorgement day	0.43 ± 0.04
3rd day after engorgement	0.46 ± 0.07
Oviposition day	7.06 ± 0.10
3rd day after oviposition	2.12 ± 0.08

Table 3 Effect of exogenous 20-E on $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity of the 3rd day after engorgement ovarian microsomal fraction

M(20-E)/($\mu\text{g}/\text{tick}$)	$\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activities ($\text{mmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$)
Control(with none)	0.96 ± 0.02
Control(with ethanol)	0.93 ± 0.03
10	1.70 ± 0.04
20	1.78 ± 0.03
40	1.75 ± 0.03

3 Discussion

In the insect, *Rhodnius prolixus*, the development of large spaces between the follicular epithelium permits the vitellogenin from the haemolymph to gain access to the oocyte surface^[5], and the development of these spaces is controlled by juvenile hormone (JH), which acts on the follicle cells to bring about a reduction in their volume^[6]. The development of the spaces and the reduction in volume of the cells is dependent on $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity.

In this study, we found that the ecdysteroid levels of haemolymph and ovary, $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity of ovarian microsomal fractions increased prior to oviposition. After oviposition, they decreased, which means endogenous ecdysteroids stimulated $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity. With regards to exogenous 20-E treatment, 20-E also stimulated its activity, but the dose response of it to various dosage of 20-E wasn't obvious, which indicated that the effect was all and no relationship^[7]. Abu-Hakima and Davey^[8] demonstrated that JH added directly to microsomal preparations of follicle cells increased the activity of $\text{Na}^+ - \text{K}^+ - \text{ATPase}$. Ilenchuk and Davey^[2] found that JH stimulated enzyme activity of vitellogenic preparations about three folds, but the activity of the other preparations (previtellogenic and postvitellogenic) was not significantly enhanced. They also demonstrated that maximal stimulations occurred at $4.0 \times 10^{-8} \sim 4.0 \times 10^{-7} \text{ mol} \cdot \text{L}^{-1}$ JH for pretreatment of microsomal preparations from vitellogenic follicles, but in this study, we found that topical application 50 $\mu\text{g}/\text{tick}$ farnesol (a JH analogue) didn't stimulate. This may indicate that there had difference in the mechanism

of uptake vitellogenin from haemolymph, we should further study their difference for elucidation their uptake mechanism.

4 References

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② 长角血蜱蜕皮激素含量与卵巢微粒体 $\text{Na}^+ - \text{K}^+ - \text{ATP}$ 酶活力的关系

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摘要 为阐明蜕皮激素在长角血蜱生殖中的作用机制, 测定了雌蜱不同生殖时期体内(卵巢和血淋巴)蜕皮激素含量和卵巢微粒体 $\text{Na}^+ - \text{K}^+ - \text{ATP}$ 酶活力的变化。结果表明, 产卵前, 蜕皮甾类含量和酶活力变化不明显; 产卵时, 则达到最高; 产卵后, 蜕皮甾类含量和酶活力又下降。外源蜕皮激素(20-羟基蜕皮酮)处理饱血3d雌蜱有激活酶活力作用, 但不表现剂量依赖关系。

关键词 长角血蜱; 生殖; 蜕皮激素; $\text{Na}^+ - \text{K}^+ - \text{ATP}$ 酶; 卵巢微粒体

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