JARQ 38 (1), 49 – 54 (2004) http://www.jircas.affrc.go.jp

REVIEW

Perspectives on Hormonal Manipulation of Shrimp Reproduction

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Abstract

Shrimp aquaculture in the world has developed remarkably, however, to enable further development, new technological advances in hormonal manipulation of shrimp reproduction are increasingly important for effective stock enhancement. To develop hormonal manipulation techniques, progress in shrimp endocrinology is necessary. In this article, I review my work and related studies on shrimp endocrinology. For female reproduction, eyestalk hormones, ecdysteroids, and vertebrate-type steroid hormones were examined. Eyestalk ablation induced ovarian development, indicating the occurrence of the eyestalk hormone, vitellogenesis-inhibiting hormone. Hemolymph levels of ecdysteroids and vertebrate-type steroid hormones were not correlated to ovarian development, suggesting that these hormones are not involved in the regulation of ovarian development. For male reproduction, the androgenic gland showed distinct structural changes in relation to male reproductive activity, suggesting that the hormone has a role in the regulation of male reproductive activity. For control of shrimp reproduction, at present, no hormones are available, and only eyestalk ablation is practically used. This is mainly because endocrinology of shrimp reproduction is not yet sufficiently understood. Only a few hormones have been detailed so far, and many hormones are still unidentified. For new technological advancements in hormonal manipulation, it is necessary to characterize these unknown hormones and to synthesize useful ones for administration.

Discipline: Aquaculture
Additional key words: androgenic gland, Crustacea, ecdysteroid, vitellogenesis

Introduction

Shrimp aquaculture in the world has developed remarkably in the last 20 years, and its annual production reached 1.27 million t (8,432 million US dollars) in 2001. For the further development of shrimp aquaculture, more efficient seed production techniques are required, and the importance of shrimp broodstock is increasing. However, at present, hormonal manipulation of shrimp reproduction is limited to eyestalk ablation for the induction of ovarian development and oviposition, because the role of individual hormones and the full diversity of hormones involved in shrimp reproduction are not yet sufficiently understood. For new technological advances in hormonal manipulation, progress in the understanding of shrimp endocrinology is essential.

I have studied shrimp endocrinology using the freshwater prawns, *Macrobrachium rosenbergii* and *Macrobrachium nipponense* and the kuruma prawn, *Marsupenaeus japonicus*. In this article, I will review my work and related studies on shrimp endocrinology and discuss perspectives on hormonal manipulation. Several detailed reviews on the recent advances and current situation of crustacean endocrinology are recommended for further reading[2–4,8,9,12,24,27].

Female reproduction

1. Vitellogenesis

In crustaceans, oocytes grow during oogenesis through the process of vitellogenesis[1,2,12]. During vitello-

This paper includes the results obtained in the 1995 short program for the cooperative research program "Development of Sustainable Aquaculture Technology in Southeast Asia" between the Japan International Research Center for Agricultural Sciences (JIRCAS), Japan and the Faculty of Fisheries in Kasetsart University, Thailand.

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Received 2 December 2003; accepted 25 December 2003.

genesis, vitellogenin, the precursor of the major yolk protein, vitellin, is synthesized and is taken in by the oocytes. In the oocytes, vitellogenin is processed and accumulated as vitellin. Vitellin is utilized as a nutritional source during embryogenesis. Vitellin and vitellogenin have been purified in several shrimps and determined to be large lipoprotein molecules (molecular weight, 280–700 kDa)[27].

During oogenesis, oocytes change histologically, and as an example, my study[17] on oogenesis in *Mac. rosenbergii* is shown in Fig. 1. Oogonia start meiotic division I and become oocytes. While the oocytes remain arrested at prophase of meiotic division I, they accumulate RNA at the previtellogenic stage, oil globules and PAS (periodic acid-Schiff)-positive vesicles at the endogenous vitellogenic stage, and yolk globules at the exogenous vitellogenic stage. At the exogenous vitellogenic stage, oocytes grow rapidly by yolk accumulation. After the completion of yolk accumulation, oocytes recommence meiosis, and the germinal vesicle at the cell center disintegrates (germinal vesicle breakdown, GVBD). Subsequently, oocytes are ovulated, and complete meiotic division by the extrusion of the first and second polar bodies.

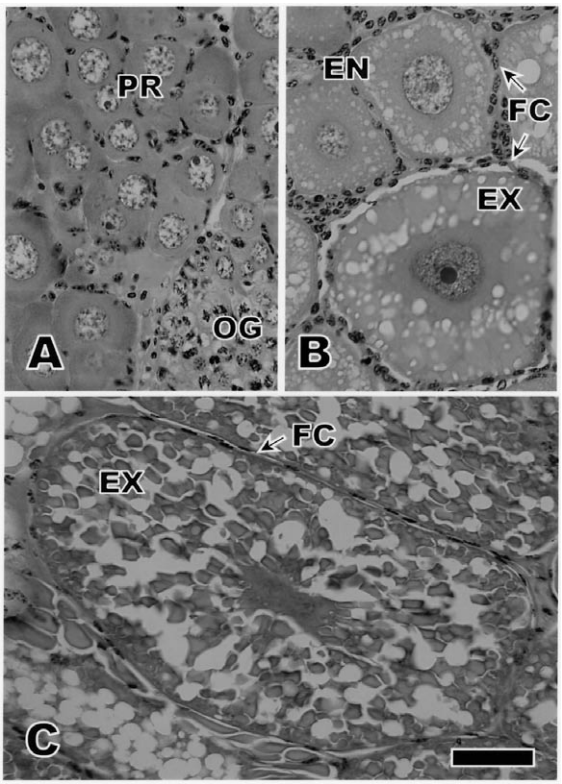

Fig. 1. Oogenesis in *Macrobrachium rosenbergii*
A: oogonia (OG) and oocytes at previtellogenic stage (PR).
B: oocytes at endogenous vitellogenic stage (EN) and early exogenous vitellogenic stage (EX).
C: oocytes at late exogenous vitellogenic stage.
FC: follicle cell. Bar = 0.05 mm.

My coworkers and I characterized vitellin and determined hemolymph vitellogenin levels in *Macrobrachium* species. Vitellin is 350 kDa, and subunits of vitellin are 102 and 90 kDa[5,17,26]. Hemolymph vitellogenin levels change in relation to gonadal development: Vitellogenin levels are high, when the gonadosomatic index (GSI) increases during molt stages C_1-D_2, and vitellogenin levels decline at oviposition between molt stages D_3-A (Fig. 2)[15,17].

Based on expression of vitellogenin mRNA, the vitellogenin synthesis sites have been determined as ovaries and hepatopancreas in *Mar. japonicus* by Tsutsui et al.[25], and as hepatopancreas in *Mac. rosenbergii* by Yang et al.[29].

2. Eyestalk hormones

It is well-known that eyestalk ablation induces ovarian development and oviposition[3,27]. This is because the source (X-organ-sinus gland complex) of the vitellogenesis-inhibiting hormone (VIH) is removed by the ablation. VIH has been purified as a peptide in the American lobster, *Homarus americanus* and in the isopod, *Armadillidium vulgare*[3,27].

My coworkers and I revealed that bilateral eyestalk ablation induced ovarian development and oviposition in *Mac. rosenbergii*[19], indicating that VIH also occurs in this species. However, VIH has not yet been identified in any shrimps.

The regulatory mechanism of VIH is partially understood. Because Khayat et al. clarified in an *in vitro* study that purified eyestalk peptides, including VIH, inhibited protein-synthesis activity of ovaries[7], VIH may

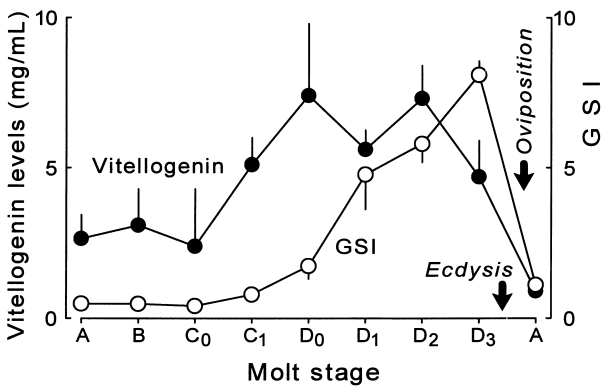

Fig. 2. Hemolymph vitellogenin levels and gonadosomatic index (GSI) in *Macrobrachium rosenbergii*
Data indicate mean and SE.

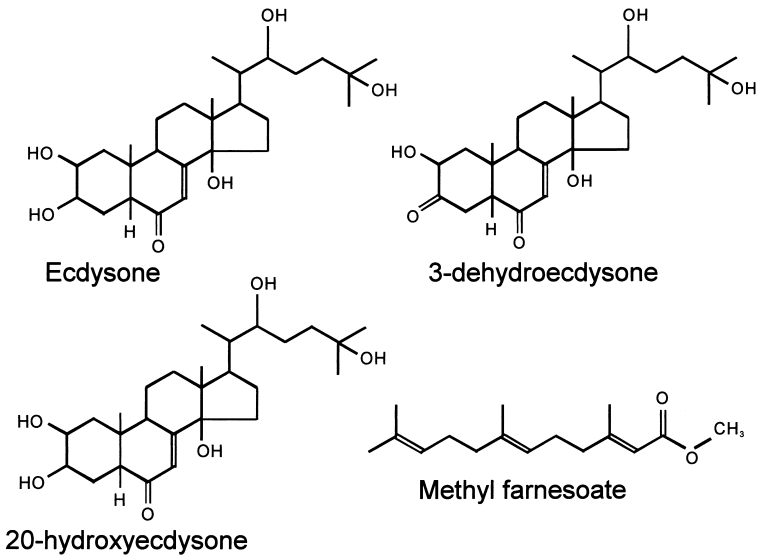

Fig. 3. Ecdysteroids and methyl farnesoate

act on the vitellogenin synthesis sites directly.

3. Ecdysteroids

Ecdysteroids are known as a molting hormone in crustaceans and insects. In crustaceans, the Y-organ produces and secretes ecdysone and 3-dehydroecdysone, and they are converted to 20-hydroxyecdysone, the biologically active ecdysteroid (Fig. 3).

Although the Y-organ had been unclear in shrimps for a long time, my coworkers and I identified the Y-organ in *Mar. japonicus* and *Mac. rosenbergii*[11,20]. During *in vitro* incubation, the Y-organ secreted ecdysteroids, mainly ecdysone and 3-dehydroecdysone. The Y-organ activity was correlated to hemolymph ecdysteroid levels[11,14,20]. This result supports the hypothesis that hemolymph ecdysteroid levels are mainly controlled by Y-organ activity.

Ecdysteroids stimulate vitellogenesis in some insects. Based on an analogy between the endocrine systems of insects and crustaceans, my coworkers and I examined the possibility that ecdysteroids work on vitellogenesis regulation in crustaceans. However, hemolymph ecdysteroid levels were not related to vitellogenesis, and showed no distinct relation to the molt cycle in *Mac. rosenbergii* (Fig. 4)[18], suggesting that ecdysteroids are not involved in vitellogenesis.

4. Vertebrate-type steroid hormones

Several vertebrate-type steroid hormones such as estradiol-17β and progesterone have been identified in crustaceans[27]. The administration of the steroids has been attempted, but the results are varied and sometimes inconsistent[27].

For a better understanding of roles of vertebrate-type steroid hormones in crustaceans, my coworker and I examined their hemolymph levels. However, significant correlations of the levels to ovarian development were not found[21], suggesting that vertebrate-type steroid hormones are not involved in ovarian development. This is supported by the recent advances in genome-wide surveys that have revealed that the genomes of the fruit fly, *Drosophila melanogaster* and the ascidean, *Ciona intestinalis* lack genes encoding the estrogen receptor or other

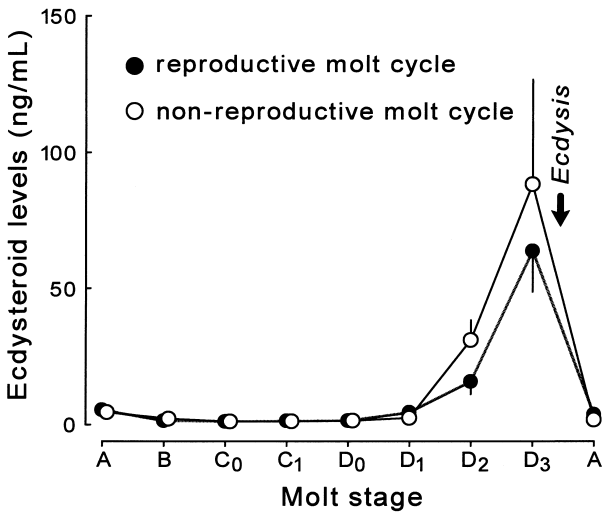

Fig. 4. Hemolymph ecdysteroid levels during the molt cycle with vitellogenesis (reproductive molt cycle) or without vitellogenesis (non-reproductive molt cycle) in *Macrobrachium rosenbergii*

Data indicate mean and SE.

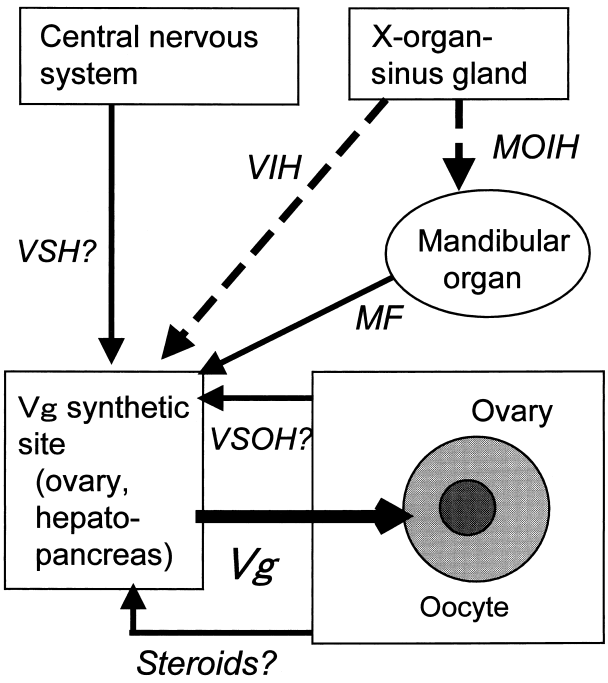

Fig. 5. Schematic diagram of the endocrine control of vitellogenesis in shrimp

MF: methyl farnesoate, MOIH: mandibular organ-inhibiting hormone, Vg: vitellogenin, VIH: vitellogenesis-inhibiting hormone, VSH: vitellogenesis-stimulating hormone.

steroid hormone receptors[28].

5. Other hormones

Methyl farnesoate (Fig. 3), crustacean juvenile hormone, stimulates ovarian development in some crustacean species[10], but does not show distinct effects in *Mac. rosenbergii*[26] nor some other species[6]. Probably, the roles of methyl farnesoate on ovarian development are not universal in crustaceans.

In addition, some undetermined factors in the brain-thoracic ganglion and ovary have been reported[3,12]. A schematic diagram of regulatory mechanism of vitellogenesis in shrimp is shown in Fig. 5.

Male reproduction

1. Spermatogenesis

In crustaceans, as in vertebrates, spermatozoa are produced through spermatogenesis[2]. Spermatogonia start meiotic division after proliferation and become spermatocytes, and spermatocytes differentiate into spermatozoa through spermatids. As an example, spermatogenesis in *Mac. rosenbergii* that my coworkers and I studied is shown in Fig. 6A-C.

2. Androgenic gland

The androgenic gland was determined to be a source of a masculinizing hormone first in the amphipod, *Orchestia gammarella*, and was characterized as a peptide hormone in *A. vulare*[23].

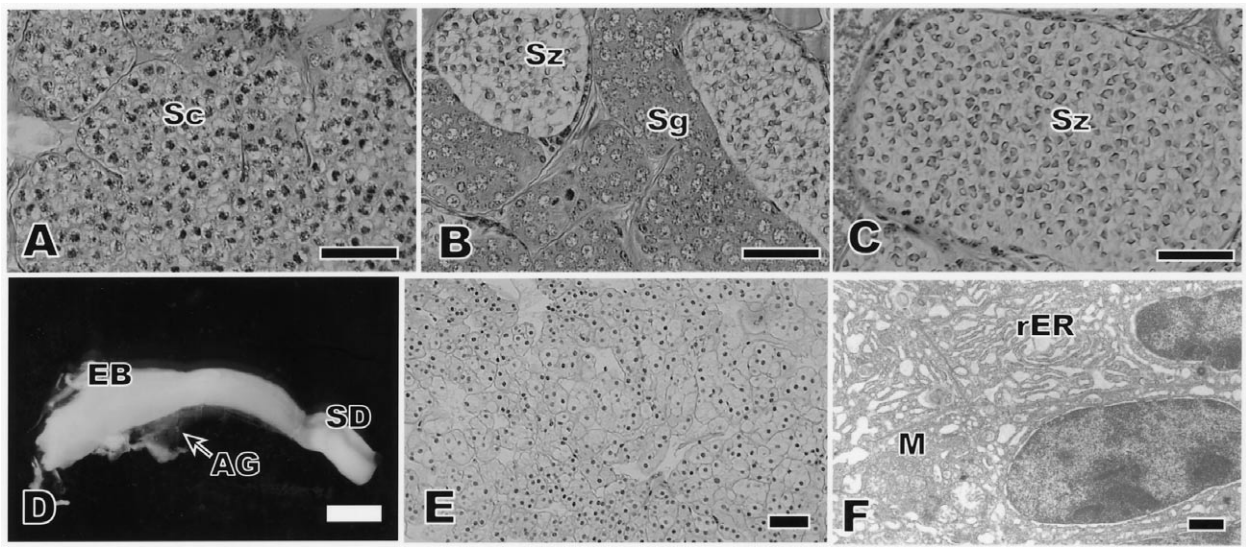

Fig. 6. Spermatogenesis (A-C) , androgenic gland (D), and cross-sections of androgenic gland (E, F) in *Macrobrachium rosenbergii*

AG: androgenic gland, EB: ejaculatory bulb, M: mitochondria, rER: rough endoplasmic reticulum, Sc: spermatocytes, SD: sperm duct, Sg: spermatogonium, Sz: spermatozoa. Bars = 0.05 mm in A-C and E, 2 mm in D, and 1 μm in F.

My coworkers and I examined roles of the androgenic gland in *Mac. rosenbergii*. In this study, samples were collected in the short program for the cooperative research program between the Japan International Research Center for Agricultural Sciences (JIRCAS), Japan and the Faculty of Fisheries in Kasetsart University, Thailand. The androgenic gland is associated with the posterior region of the sperm duct (Fig. 6D). The androgenic gland cells are rich in rough endoplasmic reticulum (Fig. 6F)[16], indicating that the androgenic gland hormone is a peptide in *Mac. rosenbergii* as in *A. vulgare*. The rough endoplasmic reticulum was developed in the reproductively active stage of male *Mac. rosenbergii*[16], suggesting that the androgenic gland hormone plays a role in the regulation of male reproductive activity.

It is known that eyestalk ablation causes hypertrophy of the androgenic gland[23]. Thus, it is considered that an unidentified hormone in the eyestalk inhibits the androgenic gland.

3. Other hormones

A schematic diagram of the regulatory mechanism on male reproduction is shown in Fig. 7. Methyl farnesoate is considered to have a function in male reproduction[10]. Some factors in the central nervous system, brain and thoracic ganglion, may act on the maintenance of spermatogonia and development of testes[2].

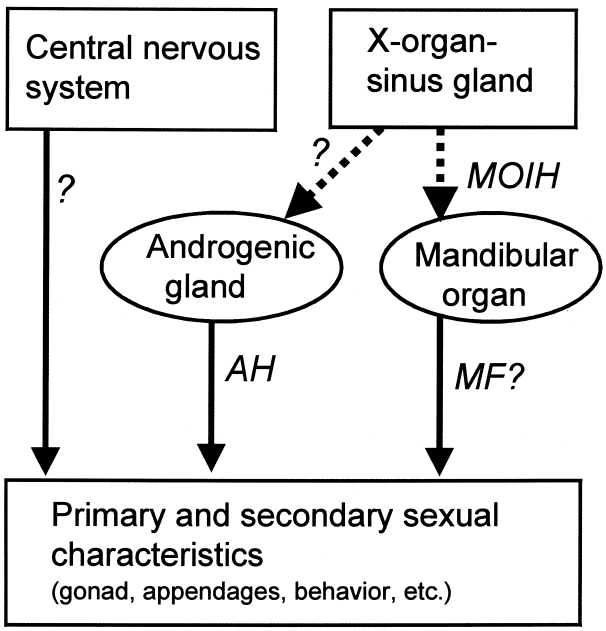

Fig. 7. Schematic diagram of endocrine control of male reproduction in shrimp

AH: androgenic gland hormone, MF: methyl farnesoate, MOIH: mandibular organ-inhibiting hormone.

However, they have not yet been characterized chemically.

Perspectives on hormonal manipulation

At present, only eyestalk ablation for the induction of female maturation is practically used in shrimp farming. This method is not repeatable and sometimes causes high mortality. If the mechanism of VIH function is fully understood, some methods for blocking VIH activity, such as anti-VIH antibody and antagonists, can be anticipated. Methyl farnesoate may be used to stimulate female maturation in the near future, however, further studies are necessary. In addition, additional stimulating factors may be newly characterized in the future. To characterize additional hormones, a suitable bioassay is required. Recently, vitellogenin was purified and its cDNA was cloned[27]. Subsequently, enzyme-immunoassay of vitellogenin and quantitative reverse-transcription (RT)-polymerase chain reaction (PCR) of vitellogenin have been developed[27]. These assay methods are very sensitive, and will be used for bioassay of the characterization process. After characterization, the factors may be synthesized by molecular biological techniques as recombinant molt-inhibiting hormone and recombinant androgenic gland hormone produced by Ohira et al.[13] and Okuno et al.[22].

For male reproduction, administration of the androgenic gland will become available, although purification of the hormone in shrimp is required. The androgenic gland hormone may be used to control sexual differentiation. In some shrimps, growth rate is different between sexes (e.g., high growth rate in males in *Macrobrachium* species and in females in Penaeid shrimps). New techniques for controlling sexual differentiation will improve economic gains in shrimp farming.

To develop hormonal manipulation techniques, much needs to be done. Only a few hormones have been discovered so far. Many hormones are still unidentified. Characterizing new hormones should be given priority. Basic studies need time and funds, but without them new technological advances will not occur.

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