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The effects of crustacean hyperglycemic hormone-family peptides on vitellogenin gene expression in the kuruma prawn, *Marsupenaeus japonicus*

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Abstract

In crustaceans, eyestalk ablation induces gonadal maturation of which vitellogenin gene expression is an essential step. However, the molecular mechanisms by which the hormones produced by the X-organ/sinus gland complex in the eyestalk regulate vitellogenesis remain poorly understood. We therefore investigated the effects of sinus gland extracts and certain sinus gland peptides belonging to the crustacean hyperglycemic hormone peptide family on vitellogenin gene expression in ovarian fragments of immature kuruma prawn, *Marsupenaeus japonicus*. Vitellogenin mRNA levels in incubated ovarian fragments were significantly higher than those in unincubated ovarian fragments prepared from the same animal. Sinus gland extracts and sinus gland peptide-III (type I peptide) both reduced vitellogenin mRNA levels in a dose-dependent manner. In contrast, neither molt-inhibiting hormone (sinus gland peptide-IV) nor molt-inhibiting hormone B, both of which are type II peptides, exerted significant effects on vitellogenin mRNA levels. These results suggest that, in the immature ovary, sinus gland peptide-III is involved in the suppression of vitellogenin gene expression. The existence of such a peptide in the X-organ/sinus gland complex provides a rationale for the significant increase in vitellogenin mRNA levels in the ovaries of eyestalk-ablated prawns.

Keywords: Neuropeptide; Ovary; Sinus gland; Vitellogenin; Vitellogenesis

1. Introduction

Vitellogenesis is an indispensable step in reproduction; oocyte development, involving the incorporation of carbohydrates, proteins, lipids, minerals, and vitamins, occurs during this process. In crustaceans, vitellogenesis is negatively controlled by a neuropeptide, vitellogenesis-inhibiting hormone (VIH), which is produced by the X-organ/sinus gland complex in the eyestalks. To date, VIH has been purified and characterized from the American lobster, *Homarus americanus*, using another shrimp species, *Palaemonetes varians*, to assay biological activity (Soyez et al., 1991), and from the terrestrial isopod, *Armadillidium vulgare*, using homologous species to assay biological activity (Gréve et al., 1999). Although these VIHs were shown to inhibit the onset of vitellogenesis, the mode of action remains unclear.

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In addition, the X-organ/sinus gland complex secretes a variety of neuropeptides, including crustacean hyperglycemic hormone (CHH), molt-inhibiting hormone (MIH), and mandibular organ-inhibiting hormone (MOIH). These peptides comprise the CHH-family due to their similarities in primary structure (Keller, 1992); most CHH-family peptides consist of 72–78 amino acid residues and contain six conserved cysteine residues which are involved in the formation of three disulfide bridges. CHH-family peptides are divided into two subtypes based on the absence (type I) or presence (type II) of a glycine residue at position 12 in mature peptide. Of the CHH-family peptides for which the function is characterized, CHH is a type I peptide, while MIH, MOIH, and VIH are generally considered to be type II peptides. There are, however, some exceptions, including type I peptides from *H. americanus* and the spiny lobster, *Jasus lalandii*, possessing both hyperglycemic and molt-inhibiting activities (Chang et al., 1990; Marco et al., 2000a), a type I peptide with molt-inhibiting activity in the crayfish, *Procambarus bouvieri* (Aguilar et al., 1996), and a type I peptide from the crab, *Libinia emarginata,* exhibiting both hyperglycemic and mandibular organ-inhibiting activities (Liu and Laufer, 1996). These exceptions demonstrate that classification into the different subtypes does not necessarily reflect peptide bioactivity.

In the kuruma prawn, *Marsupenaeus japonicus*, seven CHH-family peptides, which were designated as Pej-SGP (sinus gland peptide)-I to VII, have been purified from the sinus glands (Nagasawa et al., 1999; Yang et al., 1995, 1996, 1997). Only the type II peptide Pej-SGP-IV exhibited in vitro molt-inhibiting activity, while the other six peptides (Pej-SGP-I, II, III, V, VI, and VII), belonging to the type I subgroup displayed in vivo hyperglycemic activity. Therefore, Pej-SGP-IV is presumed to be MIH, while the remaining six peptides are considered to be CHH. In an in vitro incubation system of ovaries isolated from the green tiger prawn, *Penaeus semisulcatus*, these six peptides demonstrated a generalized inhibitory activity against protein synthesis. The proteins vitellin, major yolk protein, and shrimp ovarian peritrophin-like protein, the main component of cortical rods that emerge during the final stages of oocyte maturation, were all affected (Avarre et al., 2001; Khayat et al., 1998), implying that these six peptides are involved in the regulation of vitellogenesis. Recently, a cDNA clone encoding another CHH-family peptide, designated Pej-MIH-B, was isolated from the kuruma prawn (Ohira et al., 2005). This peptide, classified as type II, exhibited weak molt-inhibiting activity. The gene was expressed weakly in the eyestalks and strongly in the thoracic and abdominal ganglia.

Vitellin, for which vitellogenin (VG) is a precursor, is one of the many proteins sequestered in oocytes during crustacean vitellogenesis. Based on gene expression studies, the hepatopancreas and/or ovary are the synthetic sites of VG in some crustacean species (Abdu et al., 2002; Avarre et al., 2003; Chen et al., 1999; Tsang et al., 2003; Tseng et al., 2001; Tsutsui et al., 2004; Yang et al., 2000). In the kuruma prawn, the hepatopancreas and follicle cells of the ovary are responsible for VG synthesis; VG mRNA levels in those tissues and VG protein levels in the hemolymph increase significantly during vitellogenesis (Jasmani et al., 2000; Tsutsui et al., 2000). Therefore, VG mRNA levels can be employed as an index of vitellogenesis. In addition, eyestalk ablation induces gonadal maturation, including the drastic increase of VG mRNA levels in the ovary of immature prawns, suggesting that the eyestalk produces a factor that influences VG gene expression in the ovary (Tsutsui et al., 2005). Detailed examination of this phenomenon will aid in the characterization of VIH activity in the kuruma prawn and will help elucidate the regulatory mechanisms of crustacean vitellogenesis.

In this study, we investigated the effects of sinus gland extracts and CHH-family peptides on VG mRNA levels in the ovary using an in vitro incubation system, and consider aspects of the regulatory mechanisms of vitellogenesis in this context.

2. Materials and methods

2.1. Animals

Immature female kuruma prawns were purchased from a local dealer in Tokyo during the period from October to December 2003. Animals were kept in 60-L tanks and used on the day purchased. Prawns ranged from 14.1 to 19.9 g in body weight and from 0.4 to 0.7% in gonadosomatic index.

2.2. Preparation of sinus gland extracts and Pej-SGP-III, MIH, and Pej-MIH-B

The collection of sinus glands, preparation of sinus gland extracts, and purification of Pej-SGP-III were performed as described previously (Yang et al., 1995). Recombinant MIH and Pej-MIH-B peptides were prepared according to the previously reported methods (Ohira et al., 1999, 2005).

2.3. Preparation of the incubation medium

One hundred milliliters of incubation medium containing 336.8 mM NaCl, 10.7 mM KCl, 19.3 mM $MgSO_4$, 16.9 mM $MgCl_2$, 8 mM $CaCl_2$, 1 mM NaH_2PO_4, 5.4 mM $NaHCO_3$, 0.4 mM taurine (Sigma, St. Louis, MI), 2 mM glutamine (Invitrogen, Carlsbad, CA), 1.7 mM glucose, 0.01% AlbuMAX I (Invitrogen), 2 ml of MEM amino acids solution (Invitrogen, Carlsbad, CA), 1 ml of MEM nonessential amino acids solution (Invitrogen), 1 ml of