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Serotonin induces ovarian maturation in giant freshwater prawn broodstock, *Macrobrachium rosenbergii* de Man

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Abstract

This study investigated the effects of serotonin (5-hydroxytryptamine or 5HT) on ovarian development in *Macrobrachium rosenbergii* de Man. Adult female prawns at the ovarian stage I (spent) were injected with 5HT at 1, 5, 10, 20 and 50 μg g^{-1} body weight (BW) intramuscularly on days 0, 5 and 10, and sacrificed on day 15. The doses as related to the effect could be categorized into three levels: low (1 and 5 μg g^{-1} BW of 5HT), medium (10 and 20 μg g^{-1} BW of 5HT) and high (50 μg g^{-1} BW of 5HT). The low-dose, especially at 1 μg g^{-1} BW, caused prawns to exhibit a significant increase in ovarian index (ovarian weight/body weight × 100) (5.79 ± 0.09%) as compared to the control (1.49%). The ovaries of most of these prawns could develop to stage IV (mature) and contained synchronously mature oocytes while most of the control ovaries remained at stage I and II (proliferative), and contained only oogonia to previtellogenic (Oc1, Oc2) and early vitellogenic oocytes (Oc3). The medium- and high-dose treated prawns exhibited ovaries that could reach stages III and IV and contained various types of oocytes of different maturity. Pretreatment with 5HT receptor antagonist, cyproheptadine (CYP), at 10 μg g^{-1} BW before 5HT injection significantly suppressed the effect of 5HT. Intramuscular injection of the 5HT-primed thoracic ganglion culture medium into CYP-pretreated prawns resulted in the increase of ovarian index about 5–6 times more than in the control, and in the groups injected with 5HT-primed media from muscle strip, eyestalk and brain. The ovaries of most prawn could develop up to stage IV and contained synchronously developed vitellogenic (Oc4) and mature oocytes (Oc5). These findings suggest that 5HT indirectly induces ovarian development and oocytes maturation in *M. rosenbergii*, probably via a putative ovarian stimulating factor released from the thoracic ganglia.

Keywords: *Macrobrachium rosenbergii*; Broodstock; Serotonin; Oocyte; Ovarian maturation

1. Introduction

The giant freshwater prawns, *Macrobrachium rosenbergii* de Man are found in fresh as well as brackish water of the tropical area, including Southeast Asia. It is considered to be one of the crustacean species with increasing potential for aquaculture, and thus was

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introduced worldwide (Sandifer and Smith, 1985). The female prawns become sexually mature at 4–6 months, when mating occurs between hard-shelled males and soft-shelled females, which have just completed molting. A premating molted female could be distinguished by the presence of the large reddish-orange ovarian mass on the dorsal side of cephalothorax beneath the carapace. The male deposits spermatophore, a gelatinous mass containing spermatozoa, between the walking legs of the female. Egg laying occurs within a few hours and the eggs are fertilized externally. The number of eggs produced by each female is directly proportional to its size. A fully mature female may lays between 80,000 and 100,000 eggs per spawning, but at the first brood it may lay only about 5,000–20,000 eggs (New, 2002). The fertilized eggs remain attached to the brood chamber between the swimming legs for about 18–23 days after spawning. The newly spawned eggs are characterized by bright yellow to orange color, which gradually change to brown and finally grey color at about 2–3 days before hatching.

The hormonal control of decapods' reproduction was extensively studied in crayfish, *Procambarus clakii*, and lobster, *Homarus americanus*, in which a number of hormones from several neuroendocrine and endocrine organs play key roles in controlling the gonad development and secondary sexual characteristics (Van Herp and Soyez, 1997; Chen et al., 2003). At least two antagonistic neurohormones regulate the gonadal maturation: gonad inhibiting hormone (GIH), released from the sinus gland in the eyestalk optic lobes of both sexes inhibits the gonadal maturation; whilst gonad stimulating hormone (GSH), believed to be secreted by the supraesophageal and thoracic ganglia, stimulates the gonadal maturation. Several neurotransmitters have been shown to affect the release of these reproductive hormones. For instance, dopamine (DA) stimulates GIH in eyestalk (Fingerman, 1997) and inhibit GSH in thoracic ganglia (Chen et al., 2003), whereas serotonin (5HT) stimulates GSH release (Fingerman, 1997). 5HT also stimulates the releases of other hormones, including crustacean hyperglycemic hormone (CHH) (Keller and Beyer, 1968), red pigment dispersing hormone (RPDH) (Rao and Fingerman, 1970), molt inhibiting hormone (MIH) (Chang, 1985; Mattson and Spazaini, 1985) and black pigment dispersing hormone (BPDH) (Bauchau and Mangeot, 1966). In addition, it was reported that female crayfish, *Procambarus clarkii*, given 5HT exhibited a significant increase in ovarian index and oocyte size (Sarojini et al., 1995). This study aims to investigate whether 5HT could also stimulate ovarian maturation in *M. rosenbergii*, one of the most economically important prawns in Thailand. If the result is positive, the endocrine manipulation by 5HT may be one way that could be used to enhance the fecundity of female broodstock in captivity.

2. Materials and methods

2.1. Experimental animals

Adult females *M. rosenbergii* de Man were obtained from a commercial farm and they were used in the experiment as soon as they exhibited stage I (spent) of the ovarian cycle. The animals were kept in ten outdoor circular concrete tanks (1.5 m in diameter) containing water at depth 0.80 m, with adequate aeration, and 20% of water being changed daily. Commercial prawn feed was provided at 3% body weight daily. They were acclimatized under the natural light–dark cycle for 2 weeks before the experiments. To allow mating, blue-claw males were stocked in the same tank at a ratio of 1 male to 5 females.

2.2. In vivo effect of 5HT on ovarian maturation

The female animals were randomly divided into seven groups of 10 animals per group: the non-injected control, vehicle-injected control and five 5HT-injected groups. Seven female prawns with two male prawns were reared in the same tank but identified by tying different color plastic loops around their eyestalks. 5HT was dissolved in crustacean physiological saline (CPS: NaCl 29 g, KCl 0.71 g, $CaCl_2 2H_2O$ 2.38 g, $MgSO_4$ $7H_2O$ 3.16 g, $NaHCO_3$ 0.5 g, $MgCl_2 6H_2O$ 0.17 g, HEPES 4.76 g) at 10 mg ml^{-1} as the stock solution. 5HT was injected at the dosage of 1, 5, 10, 20 and 50 μg 5HT g^{-1} BW in 0.05 ml volume on the stage I of ovarian cycle. These experimental groups were designated as 5HT-1, 5HT-5, 5HT-10, 5HT-20, 5HT-50, respectively. Subsequent injections were repeated on days 5 and 10 after the first injection. Animals from each group were sacrificed on day 15. The body weight was measured on the first day, and again with the ovarian weight at the end of the experiment. Average ovarian index (OI = ovarian weight / body weight × 100) of the prawns in each group were determined. The ovaries were fixed with Davidson's fixative, and prepared for LM studies.

2.3. In vivo effect of 5HT antagonist on ovarian maturation

The other set of 70 females were divided into seven groups as in the preceding experiment. The same