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Enhancement of Vitellogenin Synthesis by Serotonin in the Giant Freshwater Prawn *Macrobrachium rosenbergii* (de Man)

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Ching-Ming Kuo, Ying-Nan Chen, Hui-Feng Fan, Hsiang-Chieh Chuang, and Shu-Ling Hsieh (2009) Enhancement of vitellogenin synthesis by serotonin in the giant freshwater prawn *Macrobrachium rosenbergii* (de Man). *Zoological Studies* **48**(5): 597-606. Serotonin (5-hydroxytryptamine, 5 HT) plays important roles in regulating diverse physiological processes in crustaceans. The stimulatory effect of 5 HT on vitellogenin (Vg) synthesis in the giant freshwater prawn *Macrobrachium rosenbergii* (de Man) is presented in this paper. During a 16 d experimental period, hemolymph was collected from both intact and bilateral eyestalk-ablated prawns 2 d after the administration of 5 HT, which was periodically injected into prawns on day 2 and every 4th day thereafter. The vitellogenin concentration in the hemolymph was quantified using an ELISA technique. The results showed that 5 HT enhanced the process of Vg synthesis in a dose-dependent manner. The fact that 5 HT is able to stimulate Vg synthesis in eyestalk-ablated prawns in a similar manner as in intact prawns, and that the synthesis and release of Vg from the hepatopancreas and the increment of total Vg mRNA in hepatopancreas were all enhanced by 5 HT stimulation in the presence of ganglion tissues *in vitro* suggest that the stimulatory action of 5 HT on Vg synthesis is mediated through a factor, likely a vitellogenesis-stimulating hormone in brain, and thoracic and abdominal ganglion tissues.
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Key words: Vitellogenin, Serotonin, Ganglion, Eyestalk-ablation, *Macrobrachium rosenbergii*.

Neurohormones are known to play important regulatory roles in reproductive processes in crustaceans, and biogenic amines are involved in the synthesis and release of various neurohormones (Richardson et al. 1991). Neurohormones involved in gonadal development and maturation include vitellogenesis-inhibiting hormone (VIH), from the X organ-sinus gland complex (Bomirsky et al. 1981, Quackenbush and Keeley 1988, Quackenbush 1989), vitellogenesis-stimulating ovarian hormone (VSOH) from follicular layers of oocytes (Takayanagi et al. 1986), vitellogenesis-stimulating hormone (VSH, also called gonad-stimulating hormone, GSH) from the brain and thoracic ganglia (Eastman-Reks and Fingerman 1984), and juvenoids (methyl farnesoate) from the mandibular organ (Laufer et al. 1993).

5-Hydroxytryptamine (5 HT, serotonin), an important biogenic amine present in the central nervous system of crustaceans (Butler and Fingerman 1983, Laxmyr 1984, Fingerman et al. 1994), appears to function as a neurotransmitter which stimulates the release of the ovary (OV)-stimulating hormone in the fiddler crab *Uca pugilator*, red swamp crayfish *Procambarus clarkii* (Richardson et al. 1991, Kulkarni and Fingerman 1992, Kulkarni et al. 1992, Lüschen et al. 1993), and other organisms. The involvement of 5 HT in crustacean reproductive processes is further

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demonstrated by its stimulatory effects on gonadal development and maturation of both sexes: testicular maturation in *U. pugilator* (Sarojini et al. 1993 1995a) and *P. clarkii* (Sarojini et al. 1995b), and OV maturation and spawning in *P. clarkii*, *Litopenaeus vannamei*, and *Penaeus monodon* (Sarojini et al. 1995c d, Vaca and Alfaro 2000, Alfaro et al. 2004, Wongprasert et al. 2006). Further, 5 HT induces OV maturation both *in vitro* and *in vivo*, and the stimulatory effect of 5 HT on OV maturation is presumably mediated by triggering GSH release from the brain and thoracic ganglia (Kulkarni et al. 1992, Sarojini et al. 1995d 1996, Vaca and Alfaro 2000). In addition, methyl farnesoate is a factor that stimulates gonadal maturation; its synthesis was shown to be inhibited by 5 HT (Laufer et al. 1993 1998); and 5 HT is therefore considered to be one of the most versatile neuroregulators with regard to the multiplicity of systems and functions that it modulates.

Although advances in elucidating the physiological functions of 5 HT were made, particularly in mammals, the physiological role of 5 HT in the neuroregulation of shrimp remains to be clarified. The objective of this study was to present the stimulatory effects of 5 HT on vitellogenin (Vg) synthesis in the giant freshwater prawn *Macrobrachium rosenbergii* (de Man) *in vivo* and *in vitro*, and the efficacy of 5 HT treatments on Vg synthesis was further compared with that induced by eyestalk ablation. It is hoped that development of an alternative technique for inducing OV maturation and spawning can be developed and consequently replace the eyestalk-ablation technique, which has been traditionally and widely used on crabs that do not spontaneously spawn in captivity or for the purpose of spawning synchronization. The success of this aspect will be of great value to the shrimp aquaculture industry.

MATERIALS AND METHODS

Animals and acclimation

Giant freshwater prawn *M. rosenbergii* were collected from an aquaculture farm in Pingtung County, southern Taiwan. Spontaneous OV maturation and oviposition of this species in captivity are well documented. Accordingly, egg-bearing females were selected and acclimated in a flow-through system under a photoperiod of 12 h light/12 h dark at a temperature of $28 \pm 1°C$ for 1 wk. Only intermolt (stage C_4) adult male prawns were used in the present study. The berried eggs were then scraped off, and both *in vivo* and *in vitro* experiments were begun 2 d after treatment to minimize variations in Vg synthesis associated with ovarian development of individual shrimp. The *in vivo* experimental prawns were tagged and individually housed in separate cages for the entire experimental period to avoid any mortality from the cannibalistic behavior of this species. The mean cephalothoracic length of the prawns was 4.37 ± 0.56 cm, and body weight was 17.56 ± 3.32 g.

Both intact and bilaterally eyestalk-ablated prawns were used in this study. In the eyestalk-ablated group, eyestalk ablation was performed simultaneously with the removal of the eggs. The cut edges of the eyestalks were sealed using a high-temperature soldering iron (solder pen, Hotery, Taipei, Taiwan). Both intact and eyestalk-ablated prawns were then subjected to periodic injections of serotonin (5 HT) at a dose of 2.5×10^{-7} moles/prawn. Serotonin (5-hydroxytryptamina creatine sulfate) was purchased from Sigma (St Louis, MO, USA) and dissolved in iso-osmotic prawn saline (450 mM NaCl, 15 mM $CaCl_2$, 10 mM $MgCl_2$, and 10 mM KCl). The dose of 2.5×10^{-7} moles/prawn of serotonin was injected into *M. rosenbergii* through the base of the chelae with a micro-syringe (Hamilton) in a 10 μL volume. *Macrobrachium rosenbergii* injected with 10 μL saline served as the controls. Stimulatory effects of 5 HT at the doses of 2.5×10^{-8} to 2.5×10^{-6} moles/prawn on hemolymph Vg contents were monitored for 20 d, and increases in the hemolymph Vg by periodic injections of 5 HT appeared to be dose dependent (Chen et al. 2003). An injection dose of 2.5×10^{-7} moles/prawn was adopted in this experiment. Samples of hemolymph were collected from prawns at the beginning of the experiment (day 0) and every 4th day thereafter during the experimental period. Injections of 5 HT were given on the 2nd day after each sampling of hemolymph. The hemolymph was extracted from the junction of the cephalothorax and abdomen, while injections were given through the sinus under the rostrum. The hemolymph Vg content from each female prawn was monitored throughout the experimental period.

The culture medium for the *in vitro* experiment was modified from that described by Hsu et al. (1995), i.e., Leibovitz's L 15 medium (1.5%) supplemented with 1% antibiotic-antimycotic (Invitrogen, Carlsbad, CA, USA) and 0.5% NaCl (pH 7.8)

with a final osmolality of 500 mOsm/kg. This culture medium was used exclusively for both the preincubation and incubation periods.

For the *in vitro* experiment, the hepato-pancreas (HP), OVs, brain, and thoracic and abdominal ganglia were extracted from each prawn biopsied. The HP and OV tissues were further sectioned, at around 0.2 g each. Each thoracic and abdominal ganglion was sectioned into 3 equal pieces, while the whole brain was used for a single incubation. To understand Vg release from HP and OV tissues of giant freshwater prawn *M. rosenbergii* under stimulation by 5 HT (i.e., serotonin) and various ganglion tissues *in vitro*, we added different ganglion tissues (brain ganglion, thoracic ganglion, and abdominal ganglion) to both the HP and OV tissues. The incubates of various combinations were preincubated for 4 h, and the experiment began then and lasted for 10 h; the culture medium was changed every 2 h. At the time of the changes, 1/2 of the culture medium (1 ml) was pipetted out and replaced with an equal volume of new medium. Vg contents in the media collected were quantified by an enzyme-linked immunosorbent assay (ELISA), and the results are presented in units of tissue wet weight. In addition, HP sections incubated under various experimental conditions *in vitro* were also collected every 2 h during the 10 h experimental period to monitor changes in total Vg RNA contents.

Vg quantification

Vg was extracted and purified from mature OVs of *M. rosenbergii* using ion exchange high-performance liquid chromatography (HPLC), and antisera against purified Vg were prepared according to procedures described by Chen and Kuo (1998). The hemolymph Vg level was measured by an ELISA technique. The hemolymph was first diluted with a buffer (15 mM Na_2CO_3 and 35 mM $NaHCO_3$ (pH 9.6) containing 0.02% NaN_3) and then was coated onto 96 well plates for 16 h at 4°C. Antiserum raised against *M. rosenbergii* vitellin and a goat anti-rabbit IgG antibody conjugated with alkaline phosphatase (Jackson Immuno Research Laboratory, West Grove, PA, USA) was sequentially applied, and finally 0.1 mg p-nitrophenyl phosphate in 10 mM diethanolamine containing 0.5 mM $MgCl_2$ (pH 9.8) was added for color development. The optical absorbance was measured using an ELISA reader (Spectra Rainbow, SLT Lab Instruments, Grodig, Austria) at a wavelength of 405 nm, and

hemolymph Vg concentrations were calculated from a standard curve established from known Vg concentrations. Net increments in hemolymph Vg levels were measured at ablation and at 5 HT injections in the eyestalk-ablated group on day 16 using the formula: [(level with the 5 HT injection-level for the control) / level with the 5 HT injection] x 100%.

Quantification of Vg mRNA

mRNA expression of the Vg gene in HP tissue incubated *in vitro* under various conditions of 5 HT and ganglia was measured by an SYBR green RT-PCR. The experimented samples were recovered each 2 h interval for the entire 10 h experimental period, and each sample was run in triplicate along with an internal control gene, β-actin.

RNA was isolated from the HP using an UltraspecTM-II RNA isolation system (Biotecx Laboratories, Houston, TX, USA) following the manufacturer's instructions. The RNA was adjusted to the same concentration with DEPC water and accurately quantified with a spectrophotometer. First-strand cDNA was synthesized using 5 μg of total RNA isolated from the HP, MMLV reverse transcriptase (Promega, Madison, WI, USA), and 50 ng of the oligo (dT) primer for 1 h at 37°C. Reaction conditions recommended by the manufacturer were followed. To quantify the prawn Vg gene, the specific primer pairs of Vg and β-actin were designed as follows: Vg forward primer, 5-GAGT CCGATCTAGCTGCAATCC-3 and reverse primer, 5-CGCACATGGCGCGCGATAG-3 (GenBank accession no.: AB056458); and β-actin forward primer, 5-CCGCCGAGCGAG-AAATC-3 and reverse primer, 5-CAATGCGACGTAGCAGA GCTT-3 (GenBank accession no.: AY626840). The SYBR green I real-time RT-PCR assay was performed in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Amplifications were performed in a 96-well plate in a 25 μl reaction volume containing 12.5 μl of SYBR Green Master Mix (Perkin-Elmer (PE) Applied Biosystems), 0.5 μl each of the forward and reverse primers (5 mM), 2 μl of the template (1 μg cDNA), and 9.5 μl of DEPC water. The thermal profile for the SYBR green real-time RT-PCR was 50°C for 2 min and 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. In a 96 well plate, each sample was analyzed in triplicate. DEPC water replaced the template as the negative control. Data analysis

of the RT-PCR was performed with SDS software vers. 2.0 (PE Applied Biosystems). The relative gene expression was quantified according to the manufacturer's instructions. Differences in the threshold PCR cycle, Ct values of the Vg gene, and the corresponding internal control, β-actin gene, were calculated and normalized for comparisons of Vg gene expression.

Statistical analysis

One-way analysis of variance (ANOVA) and Duncan's multiple-range tests were performed to determine the significance of differences among the treatments.

RESULTS

Stimulatory effects of serotonin (5 HT)

After eyestalk ablation, the hemolymph Vg titers increased from 96 to 395.9 μg/ml in saline-injected prawn and from 98.2 to 408.8 μg/ml in the 5 HT-injected group in 2 d (at day 0), with net increments of 299.9 and 310.6 μg/ml, respectively (Fig. 1A).

Hemolymph Vg titers in intact prawn increased from 96 ± 5.2 to 461.3 ± 26.97 μg/ml on day 8, with an average daily increment of 45.6 μg/ml in this initial 8 d period, followed by a continuous and substantial increase of hemolymph Vg to 1596.2 ± 55.3 μg/ml on day 16, with an average daily increment of 141.9 μg/ml in the remaining experimental period. A similar increasing trend was observed in the eyestalk-ablated group, which received saline injections. Hemolymph Vg titers increased from 395.9 ± 27.2 μg/ml on day 0 to 1665.8 ± 222.8 μg/ml on day 8, and 3498.0 ± 112.7 μg/ml on day 16. The net increments of hemolymph Vg over the 16 d period were found to be much greater in eyestalk-ablated prawn than in intact individuals. In the 5 HT injected group, increasing trends of the hemolymph Vg were nearly parallel in both the intact and eyestalk-ablated groups, and supplemental increases in hemolymph Vg with 5 HT injections were also observed. In each respective group, the additional increases due to 5 HT treatments became statistically significant ($p < 0.05$) on day 8 and thereafter. The final hemolymph Vg concentrations on day 16 in 5 HT injected prawns were 1970.2 ± 98.3 and 3949.8 ± 110.3 μg/ml for the intact and ablated groups,

respectively.

Net increments in hemolymph Vg titers were measured at 1901.8 μg/ml by ablation and at 1979.6 μg/ml by 5 HT injections in the eyestalk-ablated group on day 16. The greatest increases with supplemental injections of 5 HT into ablated prawns were found to be 300.5 μg/ml/d on days 4-8 and 321.5 μg/ml/d on days 8-12 (Fig. 1B). The overall average increments in hemolymph Vg were 93.8 and 117.0 μg/ml/d for intact prawn injected with saline and 5 HT, respectively, while for eyestalk-ablated prawn, the mean increments were 193.9 and 221.3 μg/ml/d, respectively. Accordingly, supplemental increment of Vg due to 5 HT administration was calculated to be 3.93% [(1979.6 - 1901.8 μg/ml) / 1979.6 μg/ml x 100%] for the entire experimental period.

Effects of 5 HT on the vitellogenesis of eyestalk-ablated prawn

Concentrations of Vg released from the HP *in vitro* under the influence of 5 HT at various doses of 10^{-8}-10^{-6} moles were found to be in the range of 7.71-7.80 μg/g tissue/h, which is comparable to the 7.68 mg/g tissue/h of the blank control (Fig. 2A). Supplementation with brain, thoracic, and abdominal ganglia all enhanced Vg release from the HP *in vitro* in a range of 21.5-23.4 μg/g tissue/h, and the addition of 5 HT at doses of 10^{-8}-10^{-6} moles further elevated Vg release in a dose-dependent manner, at ranges of 20.7-24.3 μg/g tissue/h at 10^{-8} mole, 26.3-28.9 μg/g tissue/h at 10^{-7} mole, and 33.5-36.6 μg/g tissue/h at 10^{-6} mole (Fig. 3). Stimulatory effects of 5 HT on Vg release from OV fragments were further examined *in vitro*. Vg released from OV tissues with no supplementation of ganglia or 5 HT was found to be 14.87 ± 0.52 μg/g tissue/h, while the release rate of Vg with the addition of 5 HT with or without various ganglia was at comparable levels of 14.48-14.83 μg/g tissue/h, which differed from the blank control (Fig. 2B).

Relative expression of the Vg gene in the HP

The designed primers for Vg and β-actin were shown to be appropriate for measuring mRNA expressions of the Vg gene in this study. The 108 and 56 bp lengths of amplified products were respectively measured by the Q-PCR for Vg and β-actin. The relative levels of mRNA expression of the Vg gene in the HP under various experimental

conditions were monitored for a 10 h period. Expression levels of Vg mRNA (expressed as $\Delta Ct = Ct_{exp.} - Ct_{\beta-actin}$) in HP tissue ranged 2.03-2.95 and 2.79-3.09 in the control (HP alone) and blank control group (HP with 5 HT stimulation), respectively. Higher levels of Vg gene expression occurred in the 2-6 h period in both cases (Fig.

5). Levels of Vg mRNA expression (ΔCt) in the HP, which was co-incubated with 5 HT and various ganglia in the first 6 h period notably increased to a level of 3.30-5.18 with brain tissue, 4.58-5.60 with thoracic ganglia, and 4.53-5.28 with abdominal ganglia.
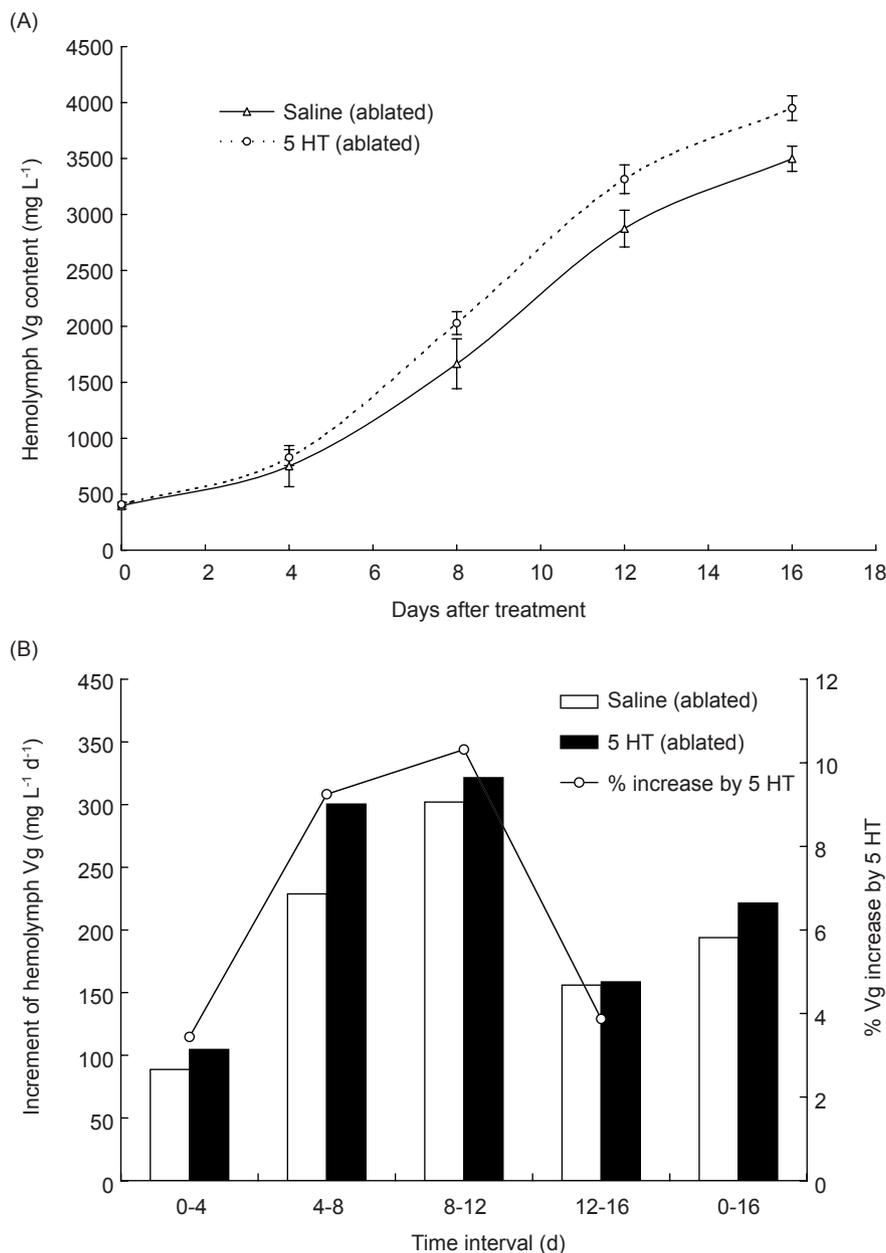
Levels of mRNA expression of the Vg gene

(A)

(B)

Fig. 1. Changes in hemolymph vitellogenin (Vg) concentrations of bilateral eyestalk-ablated giant freshwater prawn *Macrobrachium rosenbergii* receiving periodic injections of 5-hydroxytryptamine (serotonin) or saline every 4 d (panel A). Magnitudes of hemolymph Vg increases attributed to eyestalk ablation and 5-hydroxytryptamine (5 HT, serotonin) injections in *M. rosenbergii* (panel B). Day 0, 2 d after the berried eggs were removed, represents the normal control values. Each data point is presented as the mean ± SEM (*n* = 9). Both saline and serotonin (5 HT at the dose of 2.5 x 10-7 M/prawn) were injected in a 50 μl volume, every 4 d from day 2 of the 16 d experimental period.

were normalized to the control, and expression levels of Vg mRNA in the HP in ganglion-supplemented groups were found to be significantly higher than those of the blank control (HP stimulated with 5 HT alone), particularly during the 2-4 h period in which mRNA expression increased by 5.5, 7.97, and 7.53 fold with brain, thoracic, and abdominal ganglia, respectively. However, stimulation of Vg synthesis by 5 HT along with various types of ganglia was ineffective in the 6-10 h period. Similar trends of changes in the expressions of Vg mRNA in the HP and those of Vg release from the HP *in vitro* were observed.

DISCUSSION

In the 16 h experimental period, notable increases in hemolymph Vg with a daily increment of 193.9 μg/ml/d were observed in unilaterally eyestalk-ablated prawn compared to intact individuals, which showed a daily increase of 93.8 μg/ml/d hemolymph Vg in the same period. Acceleration of vitellogenesis by eyestalk ablation, attributable to removal of the vitellogenesis-inhibiting hormone, was obviously suggested. Augmentation of hemolymph Vg titers by periodic injections of 5 HT was further observed at
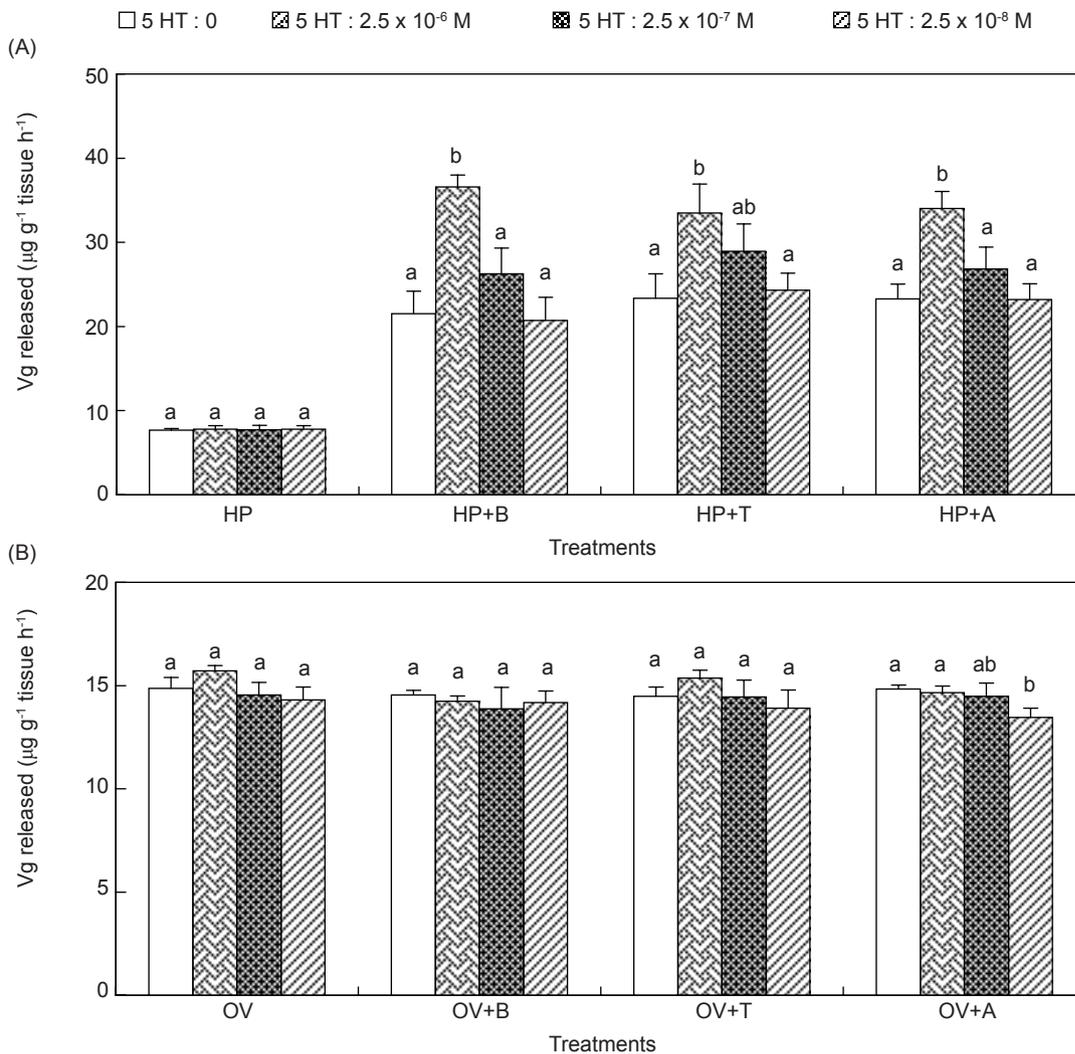

Fig. 2. Vitellogenin (Vg) release from the hepatopancreas (HP) (panel A) and ovarian (OV) tissue (panel B) of giant freshwater prawn *Macrobrachium rosenbergii* under stimulation with 5-hydroxytryptamine (5 HT, serotonin) and various ganglion tissues *in vitro*. B, brain; T, thoracic ganglion; A, abdominal ganglion. OV+B, ovarian tissue + brain ganglion; OV+T, ovarian tissue + thoracic ganglion; OV+A, ovarian tissue + abdominal ganglion. Values are presented as the mean ± SEM (n = 9). Different letters (a, b, c) indicate that differences among treatments were statistically significant at the 5% level ($p < 0.05$).

117.0 μg/ml/d for intact prawn and 221.3 μg/ml/d for eyestalk-ablated prawn. Vg synthesis reflected by changes in hemolymph Vg were prominently enhanced by eyestalk ablation, while administration of 5 HT further augmented Vg synthesis by 3.93% of the total Vg increase, and its effect varied with the treatment period, being 9.25% on days 4-8 and 10.32% on days 8-12. The predominant effect of eyestalk ablation on vitellogenesis with 5 HT treatments was obviously demonstrated in this study.

The technique of eyestalk ablation has been widely used for manipulating OV development and maturation in captivity, and is commercially practiced in shrimp hatcheries, particularly with shrimp that do not spontaneously mature and spawn. Searching for an alterative means of controlling maturation and inducing spawning is important for hatchery operations, since the method of eyestalk ablation likely disturbs normal physiological processes and produces egg-quality problems and limitations on repeated use

of broodstocks. Administration of 5 HT at 15 and 50 μg/g body wt induced OV maturation and spawning in *L. vannamei* (Vaca and Alfaro 2000), although unilateral eyestalk ablation induced a more-rapid and higher rate spawning success. The occurrence of daily spawning activity by ablation was found to be superior to that with 5 HT injections. Similarly, the process of eyestalk ablation produced more-pronounced effects on Vg synthesis in *M. rosenbergii*, while 5 HT augmented the process by as much as 3.93% of the total hemolymph Vg increase, and the percent of contribution by 5 HT varied with time during the 16 d experimental period. Hemolymph Vg titers respectively increased to 1596.2 and 1970.2 mg/L in intact prawn without and with 5 HT injections, and to 3498.0 and 3949.8 mg/L in the ablated group, in which the hemolymph Vg levels reached development stage IV (the yolk globule stage) (Chang and Shih 1995). The 5 HT injection program was therefore considered to be a practical alternative to eyestalk ablation, based on spawning
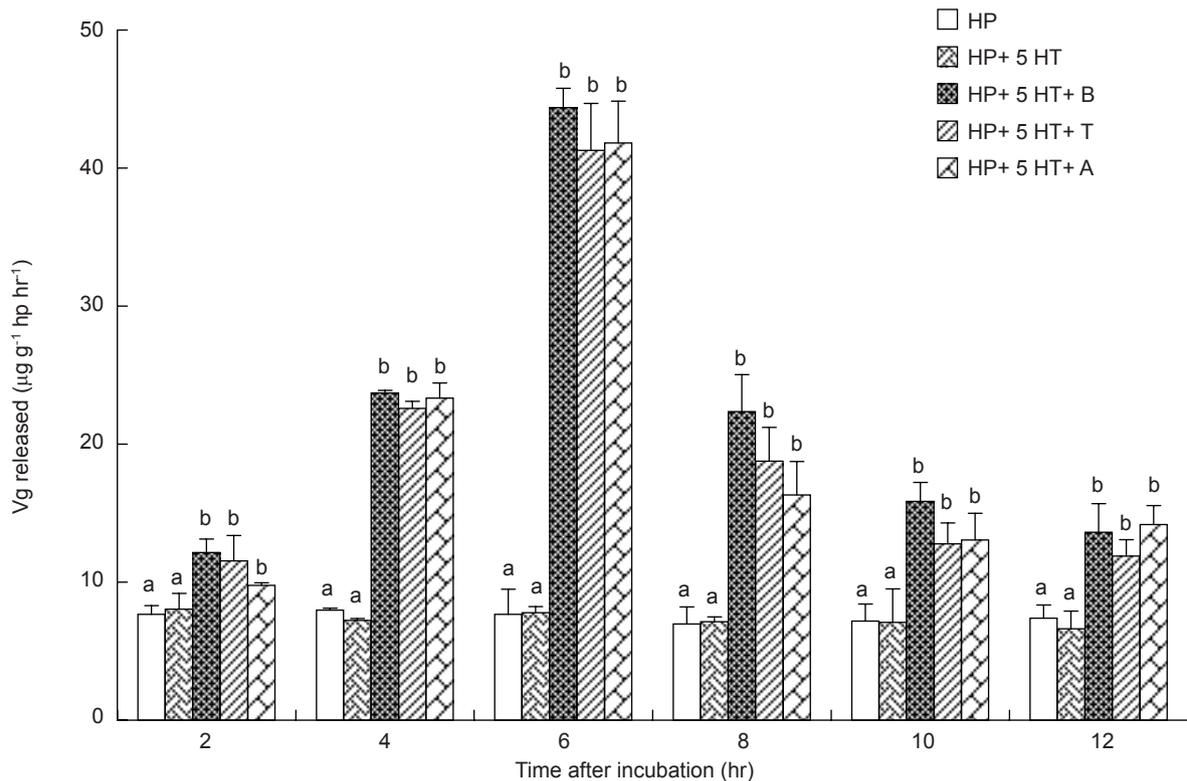

Fig. 3. Time-course changes in vitellogenin (Vg) release from the hepatopancreas (HP) of the giant freshwater prawn *Macrobrachium rosenbergii in vitro*. 5 HT, 5-hydroxytryptamine (serotonin, 10^{-6} M); B, brain; T, thoracic ganglion; A, abdominal ganglion; HP+5 HT+B, hepatopancreas + 5 HT + brain ganglion; HP+ 5 HT+T, hepatopancreas + 5 HT + thoracic ganglion; HP+ 5 HT+A, hepatopancreas + 5 HT + abdominal ganglion. Values are presented as the mean ± SEM (n = 9). Different letters (a, b, c) indicate that differences among treatments were statistically significant at the 5% level (p < 0.05).

success and egg quality (Vaca and Alfaro 2000).

Alfaro et al. (2004) induced OV maturation and spawning in *Litopenaeus stylirostris* and *L. vannamei* by combined treatment with 5 HT and the dopaminergic antagonist, spiperone. The possibility of the combined application of 5 HT and a dopamine antagonist to induce maturation is justified by the fact that dopamine is capable of suppressing vitellogenesis, while 5 HT stimulates the process (Sarojini et al. 1995c e, Chen et al. 2003).

The site of Vg synthesis has long been a subject of controversy, and the subject has often been examined by immunohistochemical and molecular approaches or tracing of isotope-labeled amino acid incorporation *in vitro*. OV tissue has been the most frequently proposed site of Vg synthesis in penaeid shrimp (Eastman-Reks and Fingerman 1985, Yano and Chinzei 1987, Quackenbush 1989, Rankin et al. 1989, Browdy et al. 1990, Shafir et al. 1992). The HP, hemocytes, and adipose tissues have also been reported as sites of Vg synthesis in crustaceans (Suzuki et al. 1989, Han et al. 1994, Chen et al. 1999, Tseng et al. 2001). In the present study, Vg was found to

be synthesized and released during the *in vitro* incubation of HP fragments of *M. rosenbergii* with various brain, thoracic, or abdominal ganglion tissues, and Vg release was further enhanced by supplementation of 5 HT in a dose-dependent manner. In contrast, higher Vg contents were initially detected in the incubation medium when OV explants of *M. rosenbergii* were incubated, and Vg contents in the medium were found to be unchanged when either nerve ganglia, 5 HT, or their combination were supplemented. These observations further confirm and substantiate a previous conclusion derived from Vg gene expression that the HP is the primary site of Vg synthesis in the freshwater giant prawn *M. rosenbergii* (Chen et al. 1999) and in white shrimp *L. vannamei* (Tseng et al. 2001).

A dose-dependent stimulatory action of 5 HT on Vg synthesis in the HP in *M. rosenbergii in vivo* was reported elsewhere (Chen et al. 2003). 5 HT, in the presence of various ganglion tissues, also showed stimulatory action on Vg synthesis *in vitro*, as indicated by increases in Vg release from the HP and the total Vg mRNA increase in the HP. In the fiddler crab *U. pugilator*, the brain and thoracic
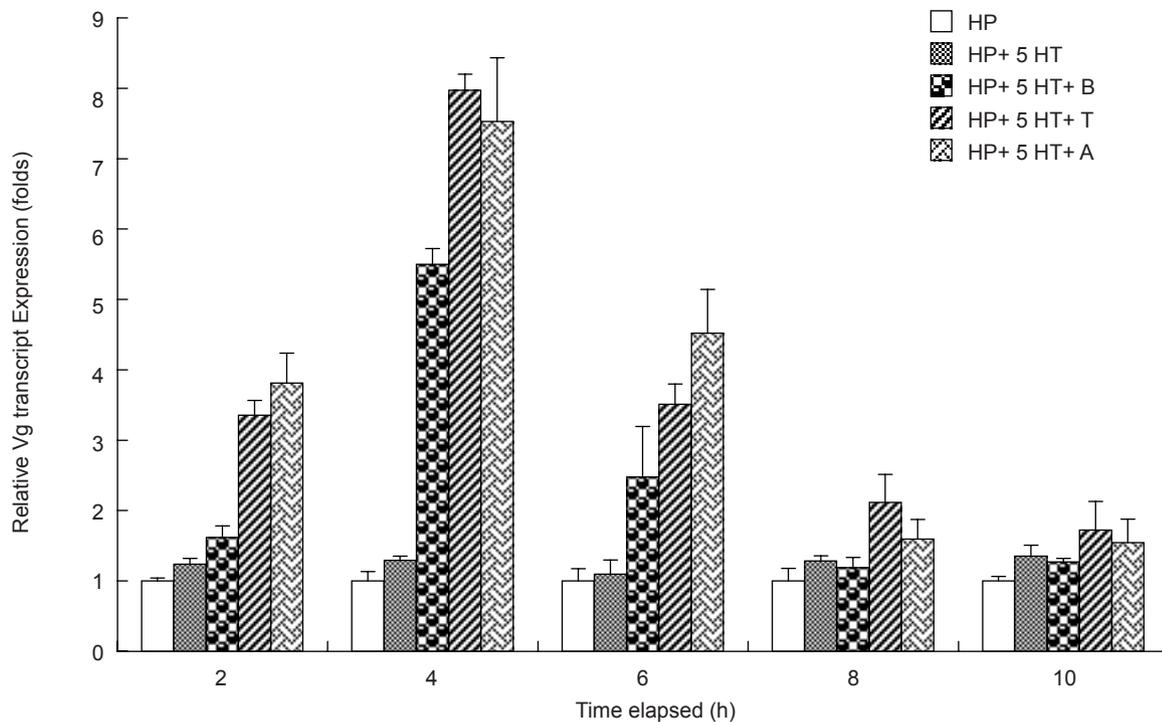

Fig. 4. Relative expression levels of the vitellogenin gene in the hepatopancreas (HP) of the giant freshwater prawn *Macrobrachium rosenbergii*. mRNA expression was measured by SYBR green RT-PCR, and each sample was run in triplicate. 5 HT, 5-hydroxytryptamine (serotonin, 10^{-6} M); B, brain; T, thoracic ganglion; A, abdominal ganglion. Values are presented as the mean ± SEM (n = 3). Different letters (a, b, c) indicate that differences among treatments were statistically significant at the 5% level ($p < 0.05$).

ganglia were shown to stimulate ovarian vitellin synthesis (Eastman-Reks and Fingerman, 1984). Similarly, leucine incorporation into OV proteins and OV maturation were stimulated by 5 HT *in vivo*, and the *in vitro* incorporation of leucine into OV proteins was observed in the presence of ganglion tissues of *P. clarkii*. (Kulkarni and Fingerman 1992, Kulkarni et al. 1992). Furthermore, 5 HT was found to induce OV maturation both *in vitro* and *in vivo* (Vaca and Alfaro 2000, Sarojini et al. 1995c d). Sarojini et al. (1995d) found that 5 HT induces OV maturation *in vivo* and *in vitro* in *Procambarus clarkia* by stimulating the release of GSH from the brain and thoracic ganglia. In *M. rosenbergii*, OV maturation was obtained by 5 HT or 5 HT-primed thoracic ganglion culture medium, and this observation suggests that 5 HT indirectly induces OV development and oocyte maturation, probably via a putative OV-stimulating factor released from thoracic ganglia (Meeratana et al. 2006). Vg release from the HP under stimulation by 5 HT was enhanced by supplementation of brain, thoracic, and abdominal ganglia *in vitro* in this study. This evidence suggests that the action of 5 HT on Vg synthesis in the HP is presumably mediated through release of a stimulatory factor, likely GSH, in various ganglion tissues, including brain, thoracic, and abdominal ganglia, although GSH is an entity which has not yet been identified nor described. Vg release was not affected by supplementation with 5 HT and various types of ganglia. These observations suggest that the HP is the primary site of Vg synthesis in this species, and Vg release is enhanced by the presence of ganglion tissues and was further supplemented by 5 HT injections in a dose-dependent manner. In contrary, 5 HT was ineffective in stimulating Vg synthesis and release in OV tissue, and the high Vg contents observed in the medium likely resulted from diffusion of the Vg constituents out of the OV tissues *in vitro*.

Diversified physiological effects of 5 HT were reported in both invertebrates and vertebrates, and its action is likely mediated through serotonergic receptors of various forms (Tiu et al. 2005). In crustaceans, 5 HT was shown to mediate many physiological processes including glucose metabolism, circadian rhythms, behavior, feeding, and reproduction (Fingerman et al. 1994, Fingerman 1997), and diverse physiological functions are positively associated with differential 5 HT receptors that were identified at the physiological and pharmacological levels (Zhang and Harris-Warrick 1994, Yeh et al. 1997,

Teshiba et al. 2001). Despite the large volume of information on the effects of 5 HT on different physiological responses of crustaceans, there is limited information on the mechanism of responses mediated by its receptors. Understanding the pathway of 5 HT's action on vitellogenesis through receptor involvement would facilitate evaluation of the potential use of 5 HT over the unilateral eyestalk ablation technique to induce maturation and subsequent spawning of shrimp.

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