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Changes in the levels, expression, and possible roles of serotonin and dopamine during embryonic development in the giant freshwater prawn, *Macrobrachium rosenbergii*

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ABSTRACT

We investigated the changes in the levels of serotonin (5-HT) and dopamine (DA), and their possible roles during embryonic development of the freshwater prawn, *Macrobrachium rosenbergii*. The 5-HT and DA concentrations were quantified using high performance liquid chromatography with electrochemical detection (HPLC-ECD). The levels of 5-HT and DA gradually increased from early developing embryos to late developing embryos. The 5-HT concentrations gradually increased from the pale yellow egg to orange egg stages, and reaching a maximum at the black egg stage. DA concentrations were much lower in the early embryos than those of 5-HT ($P < 0.05$), and gradually increased to reach the highest level at the black egg stage. Immunohistochemically, 5-HT was firstly detected in the early embryonic stages, whereas DA developed later than 5-HT. Functionally, 5-HT-treated female prawns at doses of 2.5×10^{-5}, 2.5×10^{-6} and 2.5×10^{-7} mol/prawn, produced embryos with significantly shortened lengths of early embryonic stages, whereas DA-treated prawns at all three doses, exerted its effects by significantly lengthening the period of mid-embryonic stage onwards. These results suggest significant involvement of 5-HT and DA in embryonic developmental processes of this species.

1. Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii*, is a highly valued aquatic animal for consumption that is cultured worldwide, including Thailand (Sandifer and Smith, 1985). The reproductive process and hormonal regulation of this species are not as well understood as in other decapod crustaceans, for examples, the crayfish, *Procambarus clarkii*, or the lobster, *Homarus americanus*, in which a number of neurohormones from the X-organ-sinus gland complex, situated in the eyestalks, play roles in regulating the gonadal development (Fingerman, 1997; Benton et al., 1997; Chen et al., 2003; Prasad et al., 2014). In crustaceans, the synthesis and release of neurohormones are thought to be regulated by biogenic amines, including serotonin (5-HT) and dopamine (DA) (Richardson et al., 1991; Nagaraju et al., 2010; Nagaraju, 2011). 5-HT and DA are biogenic amines that play roles in regulating various physiological processes and reproduction (Beltz, 1999). 5-HT stimulates gonadal maturation in several decapod crustaceans, including the red swamp crayfish, *P. clarkii* (Kullkarni et al., 1992), the Pacific white shrimp, *Litopenaeus vannamei* (Vaca and Alfaro, 2000), the black tiger shrimp, *Penaeus monodon* (Wongprasert et al., 2006), and the freshwater prawn, *M. rosenbergii* (Tinikul et al., 2009b), whereas DA plays an opposite role (Tinikul et al., 2014). In *M. rosenbergii*, 5-HT shortens the embryonic development period, whereas DA shows an opposite effect (Tinikul et al., 2009a). However, there is a current lack of detailed studies about the specific actions of these two neurotransmitters on each embryonic developmental stage in *M. rosenbergii*.

The levels of 5-HT and DA have been quantitated in various regions of the CNS and ovaries of many decapod crustaceans, including *Pacifastacus leniusculus* (Elofsson et al., 1982), *M. rosenbergii* (Tinikul et al., 2008), and *L. vannamei* (Tinikul et al., 2011b). Major tissues that showed high levels of 5-HT and DA activities were the ovaries, brains, and thoracic ganglia. However, the levels of these two neurotransmitters during embryogenesis in *M. rosenbergii* are still not investigated, even though there are some previous works on immunohistochemical localization of biogenic amines during embryonic development in decapod crustaceans. In the lobster, *H. americanus*, 5-HT was detected in early embryonic development, and the serotonergic neurons in the brain and ventral nerve cord are fully developed by mid-embryonic life

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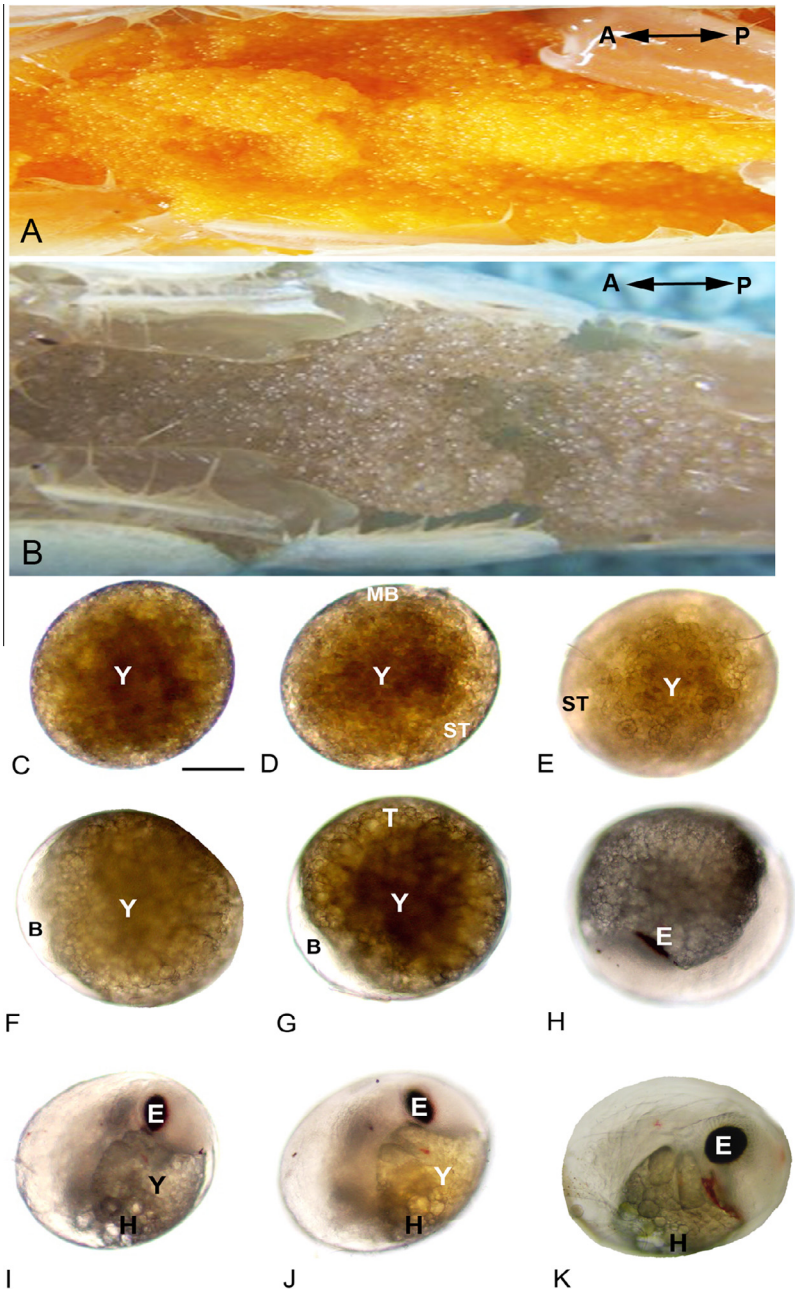

Fig. 1. Photographs of the ventral views of the brood chambers (A and B) and various stages of developing embryos (C–K) of the mature berried female *M. rosenbergii*. (A and B) The example micrographs of the ventral views of berried females at the mid-embryonic stages (orange egg stage; OE), and the late stage (gray egg stage; GE). The orientation of the prawn is given top right. (C and D) The bright yellow egg (BYE). (E) Deep yellow egg stage (DYE). (F and G) Orange egg stage (OE). (H and I) Brown egg stage (BE). (J) Gray egg stage (GE). (K) Black egg stage (BLE). A, anterior; B, blastocoel; E, eye; H, heart; MB, egg membrane; P, posterior; ST, small translucent region; T, trunk; Y, yolk. Scale bars: 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Beltz, 1999). As well, it is proposed that 5-HT may play a developmental role in this decapod crustacean, and it is likely to be related to the functions of each compound at specific life stages (Benton et al., 1997). In *H. americanus*, the DA-ir staining was first detected in the brain and subesophageal ganglion of 50% of developmental stages, indicating that DA may be involved in embryogenesis in the lobster (Cournil et al., 1995). To our current knowledge, there is no detailed study regarding the existence, distribution, and variation in the levels of these biogenic amines during embryonic development in *M. rosenbergii*.

Therefore, the aim of this study was to quantify the changes in the levels and localize the distribution of 5-HT and DA during embryonic developmental stages by using HPLC and immunohistochemistry. In addition, the functional tests were undertaken to assess the specific actions of these two neurotransmitters on each embryonic stage. The understanding how these neurotransmitters regulate embryonic development may assist in increasing the production of larvae of this species. Moreover, this study would provide valuable information to increase knowledge of evolutionary pathways of these two neurotransmitters during embryonic development in this crustacean species.

2. Materials and methods

2.1. Experimental animals

Mature female freshwater prawns (weighing on average about 32.46 ± 4.17 g) were obtained from commercial farms, in Ayuthaya