

POSSIBLE MECHANISM OF SEROTONIN INDUCES OVARIAN MATURATION IN GIANT FRESHWATER PRAWN BROODSTOCK, MACROBRACHIUM ROSENBERGII, DE MAN.

Prasert Meeratana,*** Prasert Sobhon,* Praneet Damrongphol,** Kanokphan Wongprasert,* Supatra Somapha,* Anchalee Suseangtham,* Boonsirm Withyachumnarnkul.*

*Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Road, Bangkok, Thailand 10400

** Department of Biology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok, Thailand 10400

***Department of Medical Science, Faculty of Science, Burapha University, Chonburi, Thailand

บทคัดย่อ: การวิจัยครั้งนี้ศึกษากลไกของฮอร์โมนเซโรโทนินในการกระตุ้นการเจริญเต็มวัยของรังไข่แม่พันธุ์กึ่งกำกรม โดยฉีดเซโรโทนินขนาด 1, 5, 10, 20 และ 50 ไมโครกรัม/น้ำหนักตัว 1 กรัม เข้ากล้ามเนื้อแม่พันธุ์ที่มีรังไข่ระยะ 0 ในวันที่ 0, 5 และ 10 แล้วทำการเก็บตัวอย่างรังไข่ในวันที่ 15 พบว่าเซโรโทนินขนาดต่ำ (1 และ 5 ไมโครกรัม/น้ำหนักตัว 1 กรัม) เพิ่มดัชนีมวลรังไข่ และขนาดไข่เฉลี่ยได้อย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่มควบคุม ($p < 0.05$) ขนาดกลาง และขนาดสูง (10, 20 และ 50 ไมโครกรัม/น้ำหนักตัว 1 กรัม) ให้ผลไม่แน่นอนและพบไข่หลายระยะคละกัน ไม่พัฒนาพร้อมเพรียงอย่างขนาดต่ำ การให้สารซัยโปรเฮปตาดีนยับยั้งฤทธิ์ของเซโรโทนินก่อนการฉีดเซโรโทนินให้แม่พันธุ์ พบว่าสารสามารถยับยั้งฤทธิ์กระตุ้นการเจริญของรังไข่ได้อย่างมีนัยสำคัญทางสถิติ การศึกษาด้วยการแยกกันตา สมอง ปมประสาทระดับอก และกล้ามเนื้อออกทำการเพาะเลี้ยงภายนอกร่างกายแล้วกระตุ้นด้วยเซโรโทนิน จากนั้นนำสารตัวกลางที่ใช้เพาะเลี้ยงฉีดให้แม่พันธุ์ พบว่าสารตัวกลางของปมประสาทระดับอก สามารถกระตุ้นรังไข่ให้พัฒนาเต็มวัยเร็วขึ้นได้ และการใช้สารตัวกลางเพาะเลี้ยงเนื้อเยื่อรังไข่ที่แยกจากแม่พันธุ์ 12 ชั่วโมง พบว่าขนาดเฉลี่ยของไข่ในเนื้อเยื่อรังไข่ที่เพาะเลี้ยงด้วยสารตัวกลางจากปมประสาทระดับอก เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ส่วนสารตัวกลางจากอวัยวะอื่นๆ ไม่ทำให้มีการเปลี่ยนแปลง สรุปได้ว่าเซโรโทนิน กระตุ้นการเจริญเต็มวัยของรังไข่แม่พันธุ์กึ่งกำกรมทางอ้อม โดยผ่านตัวรับกลุ่ม $5HT_{2A}$ ที่ปมประสาทระดับอก ให้หลังฮอร์โมนกระตุ้นการเจริญเต็มวัยของรังไข่

Abstract: This research was carried out to investigate the mechanisms of 5HT stimulates ovarian development in female *M. rosenbergii*. Adult prawns at ovarian stage 0 were injected with 1, 5, 10, 20 and 50 $\mu\text{g/gBW}$ of 5HT intramuscularly on day 0, 5 and 10 and sacrificed on day 15. The

prawns of low-dose 5HT treatment exhibited significant increase in ovarian index ($5.79 \pm 0.09\%$) and mean oocyte diameter ($265.23 \pm 27.29 \mu\text{m}$), ($p < 0.05$). The ovaries of prawns contained synchronously mature oocytes, while ovaries of control prawns contained previtellogenic oocytes. The medium and high doses of 5HT-treated prawns had ovaries that contained various stages of oocytes. Pretreatment with 5HT_{2A} receptor antagonist, cyproheptadine, at $10 \mu\text{g/g}$ BW, significantly suppressed the effect of low-dosed 5HT on the ovarian maturation ($2.42 \pm 0.87\%$) ($p < 0.05$). An overnight *in vitro* incubation of the ovarian explants with thoracic ganglion superfusate, but not with muscle strip, optic lobe, brain or brain/thoracic ganglia, exhibited significant increase in mean oocyte diameter ($55.06 \pm 8.55 \mu\text{m}$) ($p < 0.05$). Intramuscular injection of the thoracic ganglia superfusate, but not superfusate from other tissues, into $10 \mu\text{g/g}$ BW CYP-pretreated prawns stimulated ovarian development. These findings suggest that 5HT indirectly induces ovarian development and oocytes maturation in *M. rosenbergii*, which was via GSH releases from the thoracic ganglia. The stimulatory action was possibly mediated via 5HT_{2A} surface receptor on the neuroendocrine cells of the ventral nerve cord.

Introduction: Most species of decapod crustacean, reproduction is thought to be under regulation of various hormones. The complex interactions between several neuroendocrines and endocrine organs which play a key role in control of gonads development and secondary sexual characteristics have been identified in males as well as females. The two antagonistic neurohormones regulate crustacean gonadal maturation, GIH and GSH, were proposed (1). Several neurotransmitters have been identified to affect the release of reproductive hormone in crustacean, for instance, dopamine and serotonin (2). There are few reports concerned with research relating to the improvement of the aquaculture production via hormonal manipulation, a possible way to increase the harvest, which could be performed in many species of fishes. Thus, this study aims to elucidate whether the 5HT induces ovarian maturation and the mechanism of induction, which bearing any economic significance in the *M. rosenbergii*.

Materials and Methods: Adult female prawns obtained from a commercial farm were used as soon as they had ovarian stage 0. The *in vivo* effects of 5HT and 5HT antagonist on fecundity were performed by randomly divided the prawns into three groups of non-injection control, vehicle-injection control and 5HT-injection groups; 15 animals per group. 5HT was administered

intramuscularly by the doses of 1, 5, 10, 20 and 50 µg 5HT/gBW respectively. Another set of 70 female was performed as in previous protocol, except that the 5HT injection was 30 min preceded by 10 µg/g BW of cyproheptadine injection. Animals of each group were sacrificed on day 15.

In vitro action of 5HT on ovarian maturation; The immature ovary, optic lobes, brains, thoracic ganglia and muscle strips were incubated with 1 µg/ml of 5HT in 2 ml of Medium 199 for 30 min in darkness. The superfusate was isolated and placed into tissue culture plate containing stage 0 ovary from another female.

In vivo testing the present of GSH; The superfusate that came from the preceding experiment was lyophilized, made replicates and injected into ovarian stage 0 prawns on day 0, 5 and 10. At the end of each experiment, The body and ovarian weight were recorded and then the ovaries were fixed for LM study.

Results, Discussion and Conclusion: In marine and freshwater bivalves, 5HT has been shown to stimulate reproductive process by initiate meiotic division, germinal vesicle breakdown and spawning. Crayfish given 5HT showed a significant increase in ovarian index and oocytes diameter (3). In this study, low doses of 5HT increased ovarian index and shortened the period of ovarian maturation and embryonic development. These findings, which were supported by LM morphology of the ovaries and mean oocytes diameter, have never been reported in literature. In fiddler crab, 5HT and 5HT agonists, fenfluramine and fluoxetine, stimulated ovarian development and 5, 6-dihydroxytryptamine, a serotonergic neurotoxin inhibited it (4). Administration of 5HT at 15 and 50 µg/gBW to the white shrimp *Peneaus vannamei* also induced ovarian maturation and spawning (5). In this study, the induction of oocyte and ovarian maturation by 5HT was inhibited in the prawns preceded by cyproheptadine. It was found that extract of the brain and thoracic ganglia from crayfish, crab and shrimp stimulated ovarian vitellogenin synthesis and development of secondary oocytes, whereas the extract of the muscle tissue did not (6). It was proposed that, 5HT may act directly on the gonads or indirectly by stimulating release of GSH from the thoracic ganglion or inhibit release of GIH from the optic lobe. Evidence suggesting the direct action on the gonads is scarce, while that suggesting the indirect actions seems to be more. 5HT had been found in crayfish central nervous system, eyestalk, brain, subesophageal, thoracic, and abdominal ganglia. In this study, the ovarian tissues incubated with thoracic ganglion superfusate had a significant increase in mean oocyte diameter, the results were supported by morphological study.

It strongly suggests that 5HT did not directly induced ovarian maturation, but through its action on the thoracic ganglia. In this study, injection of superfusate from the thoracic ganglia to the ovarian stage 0 prawns induced significantly increased in ovarian index and mean oocyte diameter comparing to the control, but no significant different among other types of tissue, suggesting that 5HT acted indirectly on the ovary by stimulating GSH releases from thoracic ganglia in ventral nerve cord of the *M. rosenbergii*. Several studies have been directed at 5HT-receptors on gonadal cells of bivalves. 5HT binds to egg surface receptors, which were of different types among species. 5HT₁ receptors are divided into one that stimulates and another that inhibits adenyl cyclase. 5HT_{1A} subtype receptor inhibits adenyl cyclase via Gi protein and subsequently stimulate phospholipase C (7, 8). In crabs, CYP reduced MIH release from the optic lobe (9); it is thus possible that, the inhibition on 5HT-induced-ovarian-maturation of CYP in *M. rosenbergii* might also be an inhibition on 5HT_{2A} receptor in thoracic ganglia as well.

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Key words: Giant freshwater prawn, Broodstock, *M. rosenbergii*, Serotonin, Ovarian maturation