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EFFECTS OF 5-HT AND DA ON MOULT DURATION OF FEMALE GIANT FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII*

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ABSTRACT

In the present study effects of injection of both 5-HT and DA on total moult duration of the intact and eyestalk ablated female prawn, *Macrobrachium rosenbergii*. The total moult duration was investigated through setogenesis of uropod. After injection of 5-HT prolongs the moult duration in intact prawns, however injection of DA caused to reduce moult duration period in intact prawns. Injections of both 5-HT and DA did not caused any changes in moult duration period in eyestalk ablated prawns. In the present investigation hypothesis that both 5-HT and DA involves in the regulation of moulting.

KEYWORDS: Moult duration, moult stages, *M. rosenbergii.*

INTRODUCTION

In crustaceans molting is a cyclic process and is essential for growth, reproduction and metamorphosis. Change in form and increase in size can only occur when the hard calcareous exoskeleton is shed in crustaceans (Passano, 1960). In order to moult the species must loosen the connectives between the epidermis and extracellular cuticle, rapidly escape from their confines of the rigid exoskeleton, uptake water from the surroundings and the quickly harden with minerals and proteins for defense and locomotion. Integration and hardening of a new exoskeleton are essential parts of arthropod growth and moulting.

Eyestalk ablation, inhibit the release of molt inhibiting hormone thus allowing the molting hormone to initiate molting which allows the animal to grow. Previous studies reported that unilateral eyestalk ablation results in shortening moult interval in shrimps (Lin and Crewell, 2001) and increased weight and decreased osmolality of haemolymph (Nagabhushanam and Jyothi, 1977). Other studies also demonstrated that endocrine control of molting and growth.

In decapods crustaceans (Ponnuchamy et al., 1981). Enhanced growth rate in unilaterally eyestalk ablated freshwater prawns, *Macrobrachium lankesteri* (Cooke and Sullivan, 1985) and *M. malcolmsonii*.

(Murugadas and Mathavan, 1987). Recent data indicate the hemmolymphatic MIH titer does not strictly confirm to predictions of the model (Nakatsuji and Sonobe, 2004; Chung and Webster, 2005) and compounds other than MIH have been implicated, either directly or indirectly, in the regulation of Y-organs (Dell et al., 1999; Yu et al., 2002).

The Y-organs are negatively regulated by the molt-inhibiting hormone (MIH), which is synthesized and secreted by the XO-SG complex in the eyestalks (Chan et al., 2003). Consequently, eyestalk ablation (ESA) eliminating the primary source of MIH (Skinner, 1985) leads to enhanced ecdysteroid synthesis and secretion by Y-organs and precocious molting (Kkller and Schmid, 1979), while injection of eyestalk extract or synthetic MIH into eyestalk-ablated animals lowers the ecdysteroid titer and delays molting (Nakatsuji and Sonobe, 2004).

Vitellogenesis can inhibit molting (Lachaise et al., 1992) and eyestalk ablated animals continue to show molt cycle-dependent fluctuations in haemolymph ecdysteroid titers (Chang, 1985). *In vitro* studies indicate that MIH acts directly on Y-organs to suppress synthesis of ecdysteroids (Webster, 1986; Schkoettker and Gist, 1990) and uptake of lipoprotein-bound cholesterol, the biosynthetic precursor of ecdysteroids (Watson and Spaziani, 1985a,b; Kang and Spaziani, 1995a,b).

The cellular mechanism of action of MIH remains an area of active research. Radio receptor binding assays, using (^{125} I) MIH as ligand, indicate MIH receptors are present in Y-organ membrane preparations (Webster, 1993; Chung and Webster, 2003), but the receptor has not been isolated or thoroughly characterized for any crustacean species. Available data indicate that MIH-

receptor is linked to activation of one or more cyclic nucleotide cell signaling pathways (cAMP or cGMP, or both) (Saidi et al., 1994; Nakatsuji et al., 2006a,b). Additional cell signaling pathways, including calcium signaling, have been linked directly or indirectly to MIH action (Spaziani et al., 2001; Imayavaramban et al., 2007).

Earlier studies suggest that release of MIH is controlled by neurotransmitters. Obviously there is lack of knowledge with regard to the role of biogenic amine in the moulting of giant freshwater prawns. Hence attempts are to be made to examine the role of 5-HT and DA on the moulting in *M. rosenbergii*.

RESULTS

Results presented in this study demonstrate the effects of injection of 5-HT and DA (10^{-5} mole /prawn) on total moult duration (days) of intact and eyestalk ablated female *M. rosenbergii* during 28day experimental period.

The results clearly show that injection of 5-HT and DA significantly ($P<0.001$) increased and decreased (Table 3.1) moult duration respectively in *M. rosenbergii*. Results also revealed that the magnitude of percent change in moult duration was longer in 5-HT injected prawns than in DA injected prawns compared to controls (Fig. 3.1).

Table 3.2 presents results on the effect of injection of 5-HT and DA on the total moult duration in eyestalk ablated female *M. rosenbergii*. It is clear from the results that there was no significant variation in the moult duration of eyestalkless normal and saline injected (concurrent) controls. Results also show that moult duration did not significantly increase or decrease after injection of 5-HT and DA respectively when compared to eyestalk ablated controls.

Table 3.3 presents results pertaining to the effect of injection of serotonin and dopamine (10^{-5} moles /prawn) on the duration of each moult stage in intact prawns (from intermoult to post moult stage). It is apparent from the results that 5-HT increased the duration of each moult stage whereas DA decreased the duration compared to saline injected controls.

Table 3.4 presents results on the effect of injection of 5-HT and DA (10^{-5} moles /prawn) on the duration of each moult stage in eyestalk ablated prawns (from intermoult to post moult stage). The results clearly show that both 5-HT and DA did not cause any significance change in the duration of each moult compared to saline injected eyestalk ablated controls.

Table 2.1: Effect of injection of serotonin and dopamine (10^{-5} moles) on total moult duration (days) (in twenty eight day experimental period) of intact *M. rosenbergii*. Values are mean ± SD of six individual observations.

Treatment	Moult duration (days)
Control	21.02
Concurrent control (saline injected)	21.27
Serotonin	^{s}25.76
Dopamine	^{s}16.14

Values marked with (s) showed that are significantly different ($P<0.001$) from each other.

Table 2.2: Effect of injection of serotonin and dopamine (10^{-5} moles) on total moult duration (days) (in twenty eight day experimental period) of ablated *M. rosenbergii*. Values are mean ± SD of six individual observations.

Treatment	Moult duration (days)
Control	16.01
Concurrent control (Saline injected)	16.15
Serotonin	^{ns}16.99
Dopamine	^{ns}15.69

Values marked with (ns) showed that are not significantly different ($P<0.001$) from each other.

Table 2.3: Effect of injection of serotonin and dopamine (10^{-5} moles) on duration of each moult stage in intact *M.rosenbergii*. Values are mean ± SD of ten individual observations.

Moult stage	Moult duration (days)		
	Control	5-HT injected	DA injected
Intermoult (C)	4.36±0.149	5.16±0.149	3.03±0.110
Premoult D0	2.48±0.134	2.8±0.129	1.88±0.134
D1	3.31±0.134	3.88±0.186	2.63±0.149
D2	2.68±0.106	3.28±0.134	2.23±0.150
D3	2.91±0.106	3.71±0.165	2.41±0.157
D4	3.93±0.094	4.35±0.095	2.8±0.129
Post moult (A1, A2 & B)	1.6±0.129	2.58±0.106	1.16±0.124

Table 3.4: Injection of serotonin and dopamine (10^{-5} moles) effects on duration of each moult stage in ablated *M. rosenbergii*. Values are mean ± SD of ten individual observations.

Moult stage	Moult duration (days)		
	Control	5HT injected	DA injected
Intermoult (C)	3.03±0.110	3.23±0.179	2.96±0.179
Premoult D0	1.88±0.134	1.96±0.149	1.83±0.235
D1	2.59±0.149	2.716±0.146	2.56±0.188
D2	2.19±0.149	2.3±0.129	2.18
D3	2.4±0.157	2.51±0.177	2.33±0.188
D4	2.8±0.129	2.96±0.11	2.73±0.179
Post moult (A1, A2 & B)	1.16±0.124	1.316±0.106	1.1±0.115

DISCUSSION

Moulting is an important and on-going process of physiological change in the life history of all crustaceans**.** To moult individuals must loosen the connectives between their living tissues and the extracellular cuticle, escape from the confines of this cuticle relatively rapidly, take up water, expand the new flexible exoskeleton and then quickly harden it for defence and locomotion. The growth of crustaceans is achived through frequent moulting, which is a successive, intricate and crucial physiological procedure involved in their metamorphosis and development (Lin, 2000; Alonzo and Mange, 2000).

Moulting in crustaceans is under the control of both positively and negatively regulating hormones. Ecdysteroids secreted from YO and methyl farnesoate secreted from MO induce precocious moulting (Chang et al., 1993) whereas moulting is negatively regulated by molt inhibiting hormone (MIH) and mandibular organ inhibiting hormone (MOIH). MIH regulates the molting process by suppressing the synthesis and release of molting hormone, 20-hydroxyecdysone, by Y-organs (Rotllant et al., 2000; Okumura and Aida, 2001). MOIH inhibits the synthesis and secretion of sesquiterpenoid hormone, methylfarnesoate. MF is known to regulate molting in the crab, *O. senex senex* (Nagaraju et al., 2006; Reddy et al., 2004) and other crab species (Rotllant et al., 2000).

The total molt duration in intact female *M. rosenbergii* after injection of 5-HT and DA is presented in table 3.1. The results clearly show that there was no significant variation in moult duration in the control and hence saline injected prawns were considered as controls. However, injection of 5-HT caused a significant increase in the duration of molt cycle in intact prawns compared to control prawns (Table 3.1). 5-HT injected prawns were found to enter into premoult (D0) stage slower than control prawns (Table 3.3). 5-HT is a neurotransmitter present in the crustacean nervous system (Elofsson et al., 1982; Laxmyr, 1984) and plays an indirect role in the regulation of various physiological processes including molting in crustaceans. It stimulates the release of molt-inhibiting hormone (Mattson and Spaziani, 1985) and perhaps, decreases ecdysteroid levels in the haemolymph, lesding to an increase in moult duration. Enhanced release of MIH by 5-HT (Mattson and Spaziani, 1985) may have a positive effect on maturation because MIH inhibits the secretion of ecdysone by the Y-organ, delaying molting (Chang, 1985). However, ecdysteroids seem to play a role in ovulation and embryonic development in Crustacea (Wilder and Aida, 1995).

In the present study bilateral eyestalk ablation significantly decreased total mult duration in *M. rosenbergii*, which is comparable with the results obtained from other crustaceans. 5-HT and DA treatment has not caused changes in total moult duration in ablated prawn (Table 3.2). Sanjeevraj et al. (1997) reported that molting and growth rates were elevated in eyestalk ablated.

Prawns compared to intact prawns. In destalked *M. rosenbergii*, a rapid increase in ecdysteroid levels and a significant decrease in molting intervals in comparison with intact prawns has been reported (Okumura and Katsumi, 2001). Like in other species such as *Astacus astacus* (Huner and Lindqvist, 1984; Gydemon and Westin, 1998, *P. clarkia* (Chen et al., 1995) and *Metapenaeus dobsoni* (Venkitaraman et al., 2004), a similar relationship has been observed in *M. rosenbergii* in the present study. Venkitaraman et al. (2004) reported high mortality rate in bilaterally eyestalk-ablated and high survival rate in unilaterally ablated. Stella et al. (2000) have reported that bilateral eyestalk ablation is a very strong inducer of moulting. Average intermoult period increased with second eyestalk ablation irrespective of size group whereas Jirapom Trisak (2000) observed that intermoult periods of *P.monodon* increased with increase in the size of the experimental shrimp.

Although many evidences throw light on the hormonal control of molting in crustacean, unfortunately, until now, the role played by different endogenous biogenic amines in the regulation of molting has been elucidated in detail. Biogenic amines classical neuroregulatory molecules with wide array of biological functions (Fingerman, 1994). 5-HT is a well recognized molecule and is involved in the regulation of several physiological activities in decapods crustaceans including reproduction and glucose homeostasis (Reddy and Pusphalatha, 2007;

Tinikul et al., 2008). 5-HT is found in the neurons of all major centers of the crustacean nervous system (Elofsson et al., 1982; Elofsson, 1983; Balzer et al., 1997).

Evidence for a neurotransmitter role of 5-HT in regulating reproduction has been presented in *P. clarkii* (Sarojini et al., 1995). In view of the fact that 5-HT has been discovered in the neurosecretory cells of the eyestalks of *M. malcolmsonii* (Komali et al., 2005), it is suggested that biogenic amines trigger the release of neurohormones in *M. rosenbergii*.

Biogenic amines such as acetylcholine, glutamate, gamma-aminobutric acid, dopamine, histamine, 5-hydoxytryptamine, norepinephrine and octopamine are known to modulate the release of peptide hormones from the sinus gland. Among these only 5-HT has been reported to cause a significant increase in MIH secretion from the isolated eyestalk ganglia incubated with known amounts of neurotransmitters. Consistent with these results, ganglia exposed to 5-HT precursor 5-hydroxytryptophan (5HTP) stimulated MIH release, where as those exposed to the tryptophan hydroxylase inhibitor p-chlorophenylalanine (PCPA) or the 5-HT receptor antagonist, cyproheptadine (CPH) secreted significantly less MIH than controls. Combining 5-HT and CPH had no significant effect on MIH release. The composite results rather consistently support an interpretation that secretion of MIH by neurosecretory cells is regulated in part by input from higher order serotonergic neurons (Spaziani et al., 1994). However, injection of DA decreases MIH activity from the isolated eyestalk ganglia in the crab, *Cancer antennarius* (Matson and Spaziani, 1985c).

There are peaks and falls in ecdysteroid titers during the moult cycle (Hopkins, 1992). In general there may be one or more preliminary rises in total in total ecdysteroid titer in late intermoult and early premoult followed by a fall; then a dramatic increase to a peak preceding the moult and followed by general decline starting just before the moult and progressing to the lowest cycle levels in the immediate postmoult stage.

Moulting in crustaceans was thought to be regulated by two hormones viz. the moult inhibiting hormone and moult hormone. It is believed that the moult inhibiting hormone is produced in the eyestalk and stored in the sinus gland where as the moult hormone is produced in the Y-organ. When the eyestalks are ablated, the moult inhibiting hormone is excluded allowing the moult hormone to act. Thus the removal of eyestalks shortens the intermoult period. Similar results have been obtained by several investigators working on the endocrine control of moulting and growth in decapods crustaceans (Abramowitz and Abramowitz, 1940; Scudamore, 1947; Ponnuchamy et al., 1981).

Eyestalk ablation is a frequently adopted procedure for induced maturation of gonads and spawning. This method has also been tried on a few occasions to enhance growth in some crabs and lobsters. The crustacean eyestalk is known to have a neurohaemal function due to the presence of the XO-SG system. Excision of eyestalk is a classical endocrinological experiments to determine the function of the eyestalk neurosecretory system. Besiddes the established effect of the reproductive function, manipulation of the hormonal supply by eyestalk extirpation can bring about alterations in the physiology of the prawns (Venkitaraman et al., 2004).

Shedding of exoskeleton or moulting forms the most important metabolic event which dominates the life cycle of crustaceans (Highnam and Hill, 1979). Growth in crustaceans can be described in terms of growth factors at each moult and the duration of successive intermoult periods. The growth factor is the percentage increase in body size at the moult. The growth process basically represents a balance between wear and deterioration on one hand and repair regeneration on the other, a process, under certain conditions, can also lead to increase in body size (Aiken, 1980).

The widely accepted view of the hormonal control of molting has long been linked to the that MIH titers remain high during intermoult and reduce during premoult, thus freeing the Y-organ from inhibition, resulting in increased ecdysteroid synthesis necessary for premoult (Jegla, 1989; Webster, 1998). This long held hypothesis was tested by measurements of MIH levels during intermoult, early and late premoult. In *P. clarkii* MIH levels, measured using a very sensitive time resolved fluoroimmuno assay, were more in intermoult and dropped during early premoult. Surprisingly, subsequent pre and postmoult MIH levels were very similar to those of intermoult (Nakatsuji and Sonobe, 2004). In *C. maenas*, during late premoult and ecdysis, a remarkable and unprecedented release of MIH was observed about one day before ecdysis (D3-4) and declining rapidly before ecdysis (Chung and Webster, 2005).

Studies of Chung and Webster (2005) have shown that not only MIH release is episodic but also levels do not decline during premoult. These results contradict the long established hypothesis of moult control, whereby high levels of MIH are proposed to inhibit ecdysteroidogenesis during intermoult and falling titers of MIH in premoult subsequently directly lead to a freeing the Y-organ from the inhibitory influence of MIH, resulting in increased.

Ecdysteroidogenesis and circulating ecdysteroid titres. Ecdysteroidogenesis and circulating ecdysteroid titres. Furthermore, during late premoult, there is a massive and unprecedented release of MIH.

Bilateral eyestalk ablation not only resulted in an accelerated moult but also delay metamorphosis in larvae

by the production of extra larval stages in the shrimp, *Palaemon macrodactylus* (Little, 1969) and *Palaemonetes varians* (Le Roux, 1984); the lobster, *H. americanus* (Charmantier et al., 1988); and in the crabs, *Rhithropanopeus harisii* (Costlow, 1968) and *Sesarma reticulatum* (Freeman and Costlow, 1980).

The present results demonstrate that 5-HT and DA seem to regulate moulting indirectly by mediating the release of crustacean neurohormones. 5-HT acts indirectly on the target organ. Delay in moult cycle duration by 5-HT treatment may be due to enhanced release of MIH from the XO-SG system or inhibition of the ecdysteroid synthesis from the Y-organ. Moult acceleration in *M. rosenbergii* by DA treatment is also indirect, involving stimulation of release of ecdysteroids or inhibition of release of MIH or increased synthesis and release of MF from the mandibular organ or a combination of all.

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