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Role of combined effects of serotonin and dopamine on carbohydrate metabolism of commercial giant freshwater prawn, *Macrobrachium rosenbergii*

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Abstract

In the present study effects of both injections of serotonin and dopamine on haemolymph glucose, total carbohydrates, glycogen and phosphorylase activity in different tissues of intact and eyestalk ablated *M.rosenbergii*. The haemolymph glucose levels was increased dose dependent in between 10^{-8} and 10^{-5} mole/prawn after injection of serotonin and dopamine. While the dose 10^{-5} mole/prawn induced peak hyperglycemia in haemolymph. After injection of serotonin and dopamine significantly induced haemolymph hyperglycemia in time dependent manner. However, maximum increased haemolymph glucose levels in 90 min period. Total carbohydrates and glycogen concentrations were decreased and phosphorylase activity increased in the hepatopancreas and muscle of intact prawn. In eyestalkless prawn there was no significant difference in carbohydrates, glycogen and phosphorylase activity after treatment. Where the injection of serotonin and dopamine either directly or indirectly induces hyperglycemia.

Key-Words: Total carbohydrates, glycogen, haemolymph glucose, phosphorylase and *M.rosenbergii*

Introduction

In decapods the sinus gland is a storage organ and release principle neurosecretory peptide hormones produced by the XO-SG neurosecretory perikarya (Spaziani et al., 1994). This complex secretes Crustacean hyperglycemic hormone (CHH) consisting of a large family of neuropeptides (Chen et al., 2005; Fanjul-Moles, 2006). CHH family hormones are divided into two subfamilies based on structural and functional properties. The first type, the chromatophorotropins family, includes a red pigment-concentrating hormone (RPCH), a white pigment-concentrating hormone (WPCH) and the black pigment-dispersing hormone (BPDH). The second type, the hyperglycemic peptide family, includes hyperglycaemic hormone (CHH), molt inhibiting hormone (MIH), mandibular organ inhibiting hormone (MOIH) and vitellogenesis inhibiting hormone (VIH, also called gonad-inhibiting hormone) (Chen et al., 2005; Soyez, 1997).

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Eyestalks constitute important components of the neuroendocrine system of crustaceans. The hemolymph glucose concentration is mainly regulated by the CHH synthesized and released from XO-SG complex in the eyestalk (Abramowitz et al., 1944). Eyestalk ablation depress the CHH family peptides from XO-SG. It has been reported that, variations in the haemolymph glucose level in crustaceans (Keller and Sedlmeier, 1998) are not uniform in different species. In several species eyestalk ablation has been shown to induce hyperglycemia including *Orconectes virilis* (Mc Whinne, 1962) and *Ocypode plantitaris* (Parvathi, 1972). Conversely, hypoglycaemia was observed in some species such as *P. monodon* (Kuo et al., 1995) and *P. clarkii* (Lee et al., 2000, 2001). Thus although bulk of evidence favors hyperglycemia, there are reports favoring hypoglycaemia. This confirms that the effect of eyestalk ablation on haemolymph sugar levels is species specific.

Besides glycolysis and TCA cycle, the presence of alternative pathways for the carbohydrate metabolism has been reported in several crustacean species (Castro et al., 1994), is under the control of eyestalk CHH hormone acts as a diabetogenic factor was first

reported by Abramowitz et al. (1944). However it took long period to isolate and characterize the crustacean hyperglycaemic hormone (CHH) which plays a crucial role in the regulation of haemolymph glucose levels via mobilization of glucose from the hepatopancreas and muscle glycogen depots (Glowik et al., 1997). Glucose thus liberated either moves into the extracellular fraction or converted intracellularely to lactate via glycolysis (Santos and Keller, 1993a).

Biogenic amines and peptidergic neuroregulators have been reported to modulate the release of different neurohormones from neuroendocrine tissues (Luschen et al., 1993). 5-HT, DA, norepinephrine (NE), epinephrine (E) and octopamine (OA) are all effective in inducing hyperglycaemic response in *P. monodon* and *M. malcolmsonii* (Kuo et al., 1995 and Komali et al., 2005). In crustaceans, the biogenic amines function mainly as neurotransmitters and neuromodulators in the nervous system and some of these molecules serve as circulating neurohormones (Sneddon et al., 2000). These biogenic amines are supposed to modulate the release of CHH from XO-SG of crustaceans (Luschen et al., 1993). Hyperglycaemic effects of these biogenic amines are reported to be mediated through CHH at the eyestalk level, but the response under cold shock was not exclusively mediated through CHH in *M. rosenbergii* (Kuo and Yang, 1999).

CHH is involved in the regulation of hemolymph sugar level and also other functions such as reproduction (De Kleijn and van Herp, 1998), molting (Webster et al., 2000), lipid metabolism (Santos et al., 1997), stress response (Lorenzon et al., 2002; Santos et al., 2001) and hydromineral regulation (Serrano et al., 2003). On the other hand synthesis and release of CHH is reported to be regulated by the administration of biogenic amines. *In vivo* injection of DA and 5-HT altered carbohydrate metabolism in crustaceans by altering blood glucose levels (Kuo et al., 1995; Lee et al., 2000) suggesting the glycaemic response was caused by increased or decreased CHH release from XO-SG complex. 5-HT has been shown to enhance the release of hyperglycemic factor (s) from the isolated eyestalk ganglia as evidenced by hyperglycemic effects in several crustacean species such as *P. clarkii* (Lee et al., 2000, 2001), *O. limosus*, *C. granulata* (Santos et al., 2001), *Palaeomon elegans* (Lorenzon et al., 1999), *Squilla mantis* and *Astacus leptodactylus* (Lorenzon et al., 2004b). Conversely, injection of DA decreased haemolymph glucose levels in the crayfish, *P. clarkii*, suggesting that DA inhibited CHH release through activation of enkephalinergic neurons (Sarojini et al., 1995c). Injection of DA also induced a marked decrease in blood glucose levels in *P. elegans* and *S. mantis* (Lorenzon et al., 1999; 2004b). On the other hand injection of DA caused hyperglycemia in *C. maenas* (Luschen et al., 1993), *P. monodon* (Kuo et al., 1995) and *M. malcolmsonii* (Komali et al., 2005) implying that CHH release was enhanced. Using ELISA, (Zou et al., (2003) demonstrated a significant increase in haemolymph CHH level with increased glucose levels in DA treated *P. clarkia*. However using the same technique Lorenzon et al. (2005) demonstrated in *P. elegans* that injection of 5-HT induced a rapid and massive release of CHH from the eyestalk into the haemolymph followed by hyperglycaemia; however, DA was not found to significantly affect CHH release and hyperglycemia.

Although these studies provide evidence that injection of 5-HT and DA alter haemolymph glucose level in decapod crustaceans through CHH release, it is obvious that such studies have rarely been carried out in penaeid prawns. Although the effect of 5-HT and DA on carbohydrate metabolism has been studied in many crustacean species, information regarding the physiological functions of these drugs in freshwater aquatic species, especially in *M.rosenbergii*, is still scanty. Therefore the effect of 5-HT and DA on carbohydrate metabolism of *M. rosenbergii* need to be studied.

Material and Methods

Procurement and maintenance

The giant freshwater prawn, *Macrobrachium rosenbergii* was collected from Sathyanarayana commercial aqua farms Leburu village, nearby Mypadu, Nellore district, Andhra Pradesh, India. The experimental prawns were procured at the time of partial harvesting and transported through truck in early morning hours to the aquatic animals maintaining laboratory contains plastic containers having filtered pond water and facilitated battery operated aerators. During transportation water temperature levels were maintained 26-28°C by adding filtered aerated pond water stored. Care was taken to see that biomass-water volume was maintained at 1gm/L. As a result mortality was brought down to a minimum of 5-10% during transportation.

In the laboratory prawns exhibiting symptoms of disease, weaker and freshly moulting were discarded. Healthy and active intermoult (C4) prawns of similar size (13±1gm) were kept in the laboratory holding tanks half filled with cartridge filtered aerated tap water and maintained for a week at 26-28°C with 12hr L: 12hr D cycle for acclimatization. The tanks containing prawns were aerated so as to have 75-80% air saturation. During this period, prawns were fed *ad libitum* twice a day with standard commercial pelleted

prawn feed (CP Aquaculture India Pvt. Ltd. Chennai, India). After one hour of feeding schedule left over feed particles, excreta, and exuvia (if any) were removed by siphoning. Thirty percent of water was changed daily for better survival of prawns (Muthu, 1981).

Preparation of test chemicals

The biogenic amines, 5-hydroxytryptamine (5-HT) and dopamine (DA) were obtained as hydrochlorides from Sigma Chemical Co. (St. Louis, MO, USA). All test solutions were prepared afresh in degassed crustacean saline (Van Harreveld, 1936) before the start of each experiment to avoid oxidation. The biogenic amines were tested in concentrations ranging between 10^{-9} and 10^{-3} mole/prawn in a volume of 10µl.

Carbohydrate Metabolism

Serotonin (5-HT) and dopamine (DA) were injected into the prawns in ranging from 10^{-3} to 10^{-9} mole/prawn in a final volume of 10µl each to find the effective concentration. The injection was given through the base of the coxa of the second pair of walking legs.

The prawns were divided into eight experimental groups, each individual group consisting of six prawns. Group-1 prawns which were not treated served as normals. Group-2 prawns injected with physiological saline (Van Harreveld, 1936) served as concurrent controls. Group-3 and group-4 prawns injected with 10^{-5} mole (effective dose) serotonin and dopamine each in a final volume of 10µl/prawn served as experimental groups for the estimation of haemolymph glucose, total carbohydrate, glycogen and glycogen phosphorylase in muscle and hepatopancreas.

Individual prawns in group 5 to 8 were bilaterally ablated 2 days before the commencement of the experiments to enable metabolism of circulating crustacean hyperglycemic hormone (CHH). Prawns in group 5 served as eyestalkless normals and those in group 6 injected with crustacean saline served as eyestalkless concurrent controls. Prawns in group 7 injected with 10^{-5} mole serotonin in a final volume of 10µl and those in group 8 injected with 10^{-5} mole dopamine in a final volume of 10µl served as experimental groups for determination of hemolymph glucose, total carbohydrate, glycogen and phosphorylase activity in muscle and hepatopancreas. In all treated groups prawns were sacrificed after 90 min to collect hemolymph and body tissues for biochemical estimations depending on prior kinetic studies.

Haemolymph glucose

Haemolymph glucose was measured by using glucose oxidase-peroxidase method (Kit from Span diagnostic Ltd., India).

To 20 µl of haemolymph 1.5 ml of working glucose reagent (Glucose oxidase- peroxidase 4-amino antipyrine phenol and stabilizers) was added. The contents were mixed well and incubated at 37^0 C for 10 min followed by the addition of 1.5 ml distilled water. The red colour so developed (Quinoneimine compound) was measured at 505 nm against a reagent blank which consists all the components excluding the sample. The glucose was quantified using a standard containing 100 mg/dL. Haemolymph glucose values were expressed as mg glucose/dL of haemolymph.

Total carbohydrate (TCHO)

Total carbohydrate was estimated by the method of Carrol et al. (1956).

5% homogenate of the muscle and 2% homogenate of the hepatopancreas were prepared in 10% trichloroacetic acid (TCA). The homogenates were centrifuged at 1000g for 15 min at 4^0 C. To 0.2 ml of TCA supernatant, 4 ml anthrone reagent was added and boiled for 15 min. The colour was read at 620 nm in a spectrophotometer (Systronics UV-VIS Spectrophotometer, 108) using a blank consisting of 0.2 ml TCA and 4 ml anthrone reagent. Total carbohydrate content was expressed as mg/gm wet weight.

Glycogen

Glycogen was estimated by the method of Carrol et al. (1956).

10% homogenates of the hepatopancreas and muscle were prepared separately in 5% TCA and centrifuged at 1000 g for 15 min at 4^0 C. To one volume of TCA supernatant, 5 volumes of 95% ethanol were added and allowed to stand overnight in cold (4^0 C). After complete precipitations, the contents were centrifuged again for 15 min at 1000 g. The supernatant was discarded and the residue was dried by placing the tubes in an inverted position for 10 min at room temperature. The residue was then dissolved in 1 ml double distilled water. 5 ml anthrone reagent was added to each test tube by constant stirring. The tubes were kept in boiling water bath for 15 min and allowed to cool to room temperature. The colour was read at 620 nm in a spectrophotometer (Systronics UV-VIS Spectrophotometer, 108) against reagent blank. Values were express as mg glycogen/gm wet weight.

Glycogen phosphorylase

(1, 4-a-D-glycogen: orthophosphate a-D-glycosyl-N-transferase; EC: 2.4.1.1)

Phosphorylase activity was determined by the method of Cori et al. (1955) in the direction of glycogen synthesis by estimating the inorganic phosphate formed from glucose-1-phosphate.

5% homogenates of the hepatopancreas and muscle were prepared in aqueous medium containing 0.1M sodium fluoride and 0.037M ethylene diamine tetra acetic acid (EDTA) of pH 6.5 as suggested by Guillory and Mommaerts (1962) to avoid enzymatic inter conversion of the two phosphorylases. The homogenates were centrifuged at 1000g for 15 min. The supernatant were diluted fourfold with cysteine (0.013 M) glycerol phosphate (0.015M) buffer of pH 6.5. Then 0.4 ml of the diluted supernatant was added to 0.2 ml of 2% glycogen and the reaction was initiated by adding 0.2 ml of glucose-1-phosphate (0.016M) to one of the tubes for estimation of phosphorylase (a) activity. In addition to 0.2 ml of 2% glycogen and 0.2 ml glucose-1-phosphate, 0.2 ml of 0.04 M adenose-5-monophosphate (5, AMP) was added to the other tube to estimate total (ab) phosphorylase activity. After incubation at 37c for 30 min. and 15 min. respectively for phosphorylase "a" and "ab" 5.0 ml 10% TCA was added to each tube to stop the reaction and the contents were filtered.

The inorganic phosphate formed was estimated by the method of Fiske and Subba Rao (1925). The intensity of the blue colour was read within five min at 660 nm in a spectrophotometer against zero time controls. The enzyme activity was expressed as µ mole inorganic phosphate formed/mg protein/hr.

Results and Discussion

Figure 1.1 presents results on the effect of injection of different concentrations of serotonin and dopamine on haemolymph glucose concentrations of *M. rosenbergii*. It is clear from the results that while injection of serotonin (5-HT) and dopamine (DA) separately into intact prawns induced significant dose dependent hyperglycaemia, injection of crustacean saline did not cause any significant change in haemolymph glucose concentration. It is evident that at doses between 10^{-8} mole/prawn and 10^{-5} mole/prawn in 10µl, both serotonin and dopamine produced significant ($P<0.001$) hyperglycaemic effect. While doses lower than 10^{-8} mole/prawn, did not elicit significant hyperglycemic response, a dose of 10^{-5} mole/prawn of serotonin and dopamine caused maximal increase in haemolymph glucose concentrations compared to saline injected controls. Hence 10^{-5} mole both of 5-HT and DA in a volume of 10µl/prawn was used in the rest of the experiments. It was also interesting to note that DA, at this dose, produced significantly higher hyperglycaemia than 5-HT.

Figure 1.2 presents results on the effect of injection of both of serotonin and dopamine (10^{-5} moles in 10µl/prawn) on haemolymph glucose concentrations of *M. rosenbergii* over a period of time. The results clearly show that haemolymph glucose levels increased significantly within 30 min of injection both of serotonin and dopamine with maximum increase being recorded at 90 min and that the haemolymph glucose concentration declined gradually thereafter and returned to the basal level after 3hr. Therefore 90 min post injection time period was selected to study changes in carbohydrate metabolism.

In order to examine whether 5-HT and DA stimulate CHH release from eyestalk tissues, their effects on CHH release and glucose levels were studied in eyestalk ablated animals. Figure 1.3a presents results on the effect of injection of both serotonin and dopamine (10^{-5} moles in 10µl each; 90 min post injection time period) on haemolymph glucose concentrations of intact and eyestalk ablated (bilateral ablation) *M. rosenbergii*. As injection of physiological saline (10µl) did not cause any significant change in haemolymph glucose concentrations of intact *M. rosenbergii*, compared to normal intact prawns, saline injected prawns were taken as controls in all the ensuing experiments. The results show that bilateral eyestalk ablation caused a significant decrease ($P<0.001$) in haemolymph glucose concentrations.

The results also show that injection of 5-HT and DA (10^{-5} moles/prawn) caused a significant ($P<0.001$) increase in the haemolymph glucose concentration of intact *M. rosenbergii* at 90 min post injection compared to saline injected controls. On the other hand no significant variation was observed in the haemolymph glucose level of eyestalkless prawns after injection of 5-HT and DA (Fig. 1.3a). It was interesting to note that the percent increase in the haemolymph glucose level at 90 min post injection was more in dopamine treated prawns than in serotonin treated ones (Fig. 1.3b).

Table 1.1 and 1.2 present results on the effect of injection both of serotonin and dopamine (10^{-5} moles in 10µl each; 90 min post injection duration) on total carbohydrate (TCHO) and glycogen concentrations in the hepatopancreas and muscle of intact and eyestalk ablated *M. rosenbergii*. The results clearly show that TCHO and glycogen concentrations in the hepatopancreas and muscle of serotonin and dopamine injected intact prawns were significantly lower ($P<0.001$) than those in the hepatopancreas and muscle of saline injected control prawns. Decrease in muscle TCHO and glycogen concentrations following injection of 5-HT and DA, suggests a possible breakdown and mobilization of these macromolecules into the

haemolymph as evidenced by increased haemolymph glucose levels (Fig. 1.3a). It is also clear that the magnitude of decrease in TCHO and glycogen concentrations in the hepatopancreas and muscle of intact prawns (Fig. 1.4, 1.5) was more with dopamine than with serotonin. The results also show that bilateral eyestalk ablation caused a significant increase in TCHO and glycogen concentrations in the hepatopancreas and muscle of *M. rosenbergii*. However, there was no significant change in TCHO and glycogen concentrations of these tissues in serotonin and dopamine injected eyestalkless prawns, compared to those in eyestalk ablated controls (Table 1.1, 1.2).

Table 1.3 and 1.4 present results on the effect of injection of 5-HT and DA (10^{-5} mole/prawn; 90 min post injection duration) on the activity levels of phosphorylase ' a' (active) and ' ab' (total) in the hepatopancreas and muscle of *M. rosenbergii* respectively. It is clear from the results that there was a significant increase ($P<0.001$) in the activity levels of phosphorylase
(' a' and ' ab') in the hepatopancreas (Table 1.3) and muscle (Table 1.4) of intact prawns after injection of 5-HT and DA compared to saline injected controls. The ratio of active to total phosphorylase also increased in both the tissues after 5-HT and DA injections (Table 1.3 and 1.4), suggesting possible conversion of inactive phosphorylase to active phosphorylase. It is also clear that the magnitude of increase in phosphorylase (' a' and ' ab') activity levels in both hepatopancreas and muscle was higher in DA treated prawns than in 5-HT treated ones (Fig. 1.6, 1.7).

The results clearly show that bilateral eyestalk ablation caused a significant decrease ($P<0.001$) in the activity levels of phosphorylase (' a' and ' ab') in the hepatopancreas and muscle of prawns, with a concomitant increase in tissue TCHO and glycogen concentrations (Tables 1.1-1.4) and a significant decrease ($P<0.001$) in haemolymph glucose concentrations (Fig. 1.3a). Interestingly injection of 5-HT and DA separately into eyestalk ablated prawns did not cause any significant change in the activity levels of phosphorylase in the hepatopancrease and muscle compared to eyestalkless controls (Table 1.3, 1.4).

Obviously an increase in phosphorylase activity, decrease in glycogen and TCHO levels of the hepatopancreas and muscle and an increase in haemolymph sugar levels of *M. rosenbergii*, injected with 5-HT and DA, suggest glycogenolysis and mobilization of sugar molecules from tissues to haemolymph.

The results of this study show that 5-HT and DA played an important role in the control of carbohydrate metabolism in the giant freshwater prawn, *M. rosenbergii*. For instance 5-HT and DA stimulate hyperglycaemia in *M. rosenbergii* in a dose dependent (Fig. 1.1) and time dependent (Fig. 1.2) manner. Apparently earlier studies are in agreement with the results of the present study. Escamilla-Chimal et al. (2001) and Santos et al. (2001) have demonstrated that serotonin, in a dose dependent manner, could enhance the release of CHH from the optic ganglia of crayfish and crabs. Lorenzon et al. (2004) have shown that 5-HT elevated blood glucose in *Palaemon elegans*, *Astacus leptodactylus* and *Squilla mantis*. Similar results have also been obtained in *M. malcolmsonii* (Komali et al., 2005) and *M. rosenbergii* (Zou et al., 2003). At 10^{-5} moles/prawn (in 10µl) concentration 5-HT and DA caused maximal increase in haemolymph glucose concentration compared to saline injected controls. Surprisingly DA, at this dose, caused a greater increase in haemolymph glucose than 5-HT (Fig. 1.1).

The effects of 5-HT, a potent hyperglycaemic factor have long been documented in several crustacean species (Luschen et al., 1993). That 5-HT is involved in the regulation of haemolyph glucose concentration, possibly controlling the release of neurohormones from the X-organ sinus gland complex, has repeatedly been demonstrated (Garcia and Arechiga, 1998). *In vivo* administration of 5-HT resulting in significant hyperglycaemia (Luschen et al., 1993) seems to be mediated by the release of CHH. Direct supporting evidence for this hypothesis was provided by Lee et al. (2001), in which it was shown that *in vivo* incubated eyestalk ganglia, when treated with 5-HT released sufficient quantities of hyperglycaemic factor into incubation medium as evidenced by a bioassay. This is in accordance with previous reports indicating an increase in the firing rate and induction of action potential by 5-HT in neurosecretory cells of the X-organ (Saenz et al., 1997), even though CHH producing cells were not specifically identified. Lorenzon et al. (2005) using ELISA, have demonstrated in *P. elegans* that injection of 5-HT induced a rapid release of CHH from the eyestalk into the hemolymph resulting in hyperglycemia.

Results of the present study clearly show that DA caused significant elevation of haemolymph glucose levels in a dose dependent manner in *M. rosenbergii*. DA was identified as a stimulator and modulator of CHH which is responsible for hyperglycaemia in crustaceans
(Komali et al., 2005). In addition, DA can induce hyperglycaemia in *O. limosus* and the shore crab,

Carcinus maenas (Keller and beyer, 1968; Luschen et al., 1993), the tiger prawn, *Penaeus monodon* (Kuo et al., 1995), and the giant freshwater prawn, *M. rosenbergii* (Kuo and yang, 1999). Lee et al. (2001) quantified DA released by CHH using sandwich ELISA in *P. clarkii*. In *M. rosenbergii* which had received DA, the haemolymph glucose levels significantly increased at 90 min and returned to normal level after an hour. This is evidently similar to the response in *L. vannamei* which received DA (Chiu et al., 2006). Physiological effects of dopamine, widely distributed in crustaceans, have previously been reviewed by Tierney et al. (2003). DA has been reported to involve in ionic and osmotic regulation in freshwater and marine crustaceans (Morris, 2001). Previous studies indicated that DA induces transient modulation of physiological responses, depresses the immune ability, and increases susceptibility to *Vibrio alginolyticus* in whiteleg shrimp, *Litopenaeus vannamei* (Cheng et al., 2005; Chiu et al., 2006). Lee et al. (2005) also reported that DA depresses the immune ability and increases susceptibility to *Lactococcus garvieae* in the freshwater giant prawn, *M. rosenbergii*.

Studies on different species have reported contrasting results with DA. In *P. clarkii* and *P. elegans* (Sarojini et al., 1995), *A. leptodactylus* and *Squilla mantis* (Lorenzon et al., 1999, 2004a), DA inhibits the release of CHH from the SG, causing a decrease in hemolymph glucose level. However injection of DA into eyestalkless animals is ineffective. By contrast, DA was shown to elevate haemolymph glucose in the crab, *C. maenas* (Luschen et al., 1993) and in the tiger shrimp, *P. monodon* (Kuo et al., 1995). More recently, Zou et al. (2003) demonstrated a dose dependent stimulating effect of DA on CHH and hemolymph glucose of *P. clarkii*. Sarojini et al. (1995), in *P. clarkii*, reported that dopamine (DA) and Leucine-enkephalin inhibit the release of CHH from the sinus gland, causing a decrease in haemolymph glucose level *in vitro* suppressing the release of CHH from the eyestalk neuroendocrine tissue.

Diabetogenic action of CHH was also documented in crustacean species (Kuo et al., 1995; De Kleijn et al., 1998; Van Herp, 1998; Kuo and Yang, 1999; Chang et al., 1999, Mettulio et al., 2004). The physiological action of the CHH released from the sinus gland have been extensively studied in the past, since its discovery by Abramowitz et al. (1944). The results suggest that both 5-HT and DA were found to mimic the action of CHH, i.e., elevating the haemolymph glucose level, with DA being more potent than 5-HT.

The hyperglycemic effects induced by DA and 5-HT could be due to triggering the release of CHH from the sinus gland of the eyestalks (Lee at al., 2000; Zuo et al., 2003). The results obtained in the present study are in consonance with reports of Komali et al. (2005) which also suggest that DA and 5-HT induced hyperglycaemia is mediated through CHH in *M. malcolmsonii*. Recent reports of Sathyanandam et al. (2008) also suggest that 5-HT induced hyperglycaemia is mediated through the release of CHH in the Indian white shrimp, *Fenneropenaeus indicus*.

Results presented in this study clearly show that bilateral eyestalk ablation caused significant hypoglycaemia, which is in concurrence with earlier findings in *M. malcolmsonii* (Komali et al., 2005). It has been reported that haemolymph sugar levels decreased in *Uca lactea annulipes* (Nagaraju and Reddy, 2002). Similar results were also obtained in *P. elegans* (Lorenzon et al., 1999) and *M. monocerus* (Kishori et al., 2001).

Bilateral eyestalk ablation did not induce hyperglycaemia in *M. rosenbergii* after injection of 5-HT and DA (Fig. 1.3), indicating an indirect mode of action of biogenic amines. These results are in agreement with the results obtained in *C. maenas* (Luschen et al., 1993), *P. monodon* (Kuo et al., 1995), *M. rosenbergii* (Kuo and Yang, 1999), and *P. clarkii* (Lee et al., 2001). Results of the present study suggest that 5-HT and DA are the potent stimulators of CHH release, which in turn enhances haemolymph glucose levels by decreasing TCHO and glycogen levels.

Results presented in tables 1.1 to 1.4 show that 5-HT and DA are involved in the regulation of carbohydrate metabolism in *M. rosenbergii*. A significant upsurge ($P< 0.001$) in phosphorylase (' a' and ' ab') activity (Fig. 1.6 and 1.7) and a decrease in TCHO and glycogen concentrations in the hepatopancreas and muscle (Fig. 1.4 and 1.5) of *M. rosenbergii* followed by hyperglycaemia (Fig. 1.3) indicate glycogenolysis and mobilization of sugar molecules from tissues to haemolymph. This is an agreement with other findings (see review by Reddy and Ramamurthy, 1999). Though the hormone that elevates haemolymph sugar is conventionally called crustacean hyperglycemic hormone (CHH), Hohnke and Scheer (1970) suggested that the primary function of the CHH is not to elevate haemolymph sugar level, but to elevate intracellular glucose through the degradation of glycogen by activating the enzyme phosphorylase. The conversion of phosphorylase from its inactive form to active form results in glycogenolysis, and the resultant glucose molecules leak into the haemolymph, causing hyperglycemia. This view has been supported by

Telford (1975). The hyperglycemic activity of CHH through the elevation of glucose level in the haemolymph is presumably exerted by stimulation of glycogen breakdown, a reaction mediated by glycogen phosphorylase, and by inhibitory effect on glycogen synthase (Sedlmeier, 1982).

Bilateral eyestalk ablation reverses the effects of both 5-HT and DA by enhancing the release CHH from the XO-SG organ. This findings reinforces previous ideas on suggesting that hyperglycaemia results from enhanced release of CHH located in the XO-SG (Fingerman, 1997; Lee et al., 2001; Zou et al., 2003). The interaction of monoamines with putative extraocular CHH secretion locus of *Homarus* has been proved by means of immunoreactive, confocal imaging and electrophysiological methods (Basu and Kravitz, 2003). Both 5-HT and dopamine inhibit the spontaneous bursting of CHH immunoreactive neurons of the second thoracic nerve roots. Although much remains to be determined about the regulation, synthesis and release of CHH, there is evidence to show that glucose uptake and glycogenesis in muscle and mid gut gland of the crab and lobster may be under hormonal control by insulin-like peptides (Chang, 1995; Richardson et al., 1997).

Results of the present study provide evidence for the involvement of DA and 5-HT in the regulation of carbohydrate metabolism in the freshwater prawn, *M. rosenbergii*. The results also suggest that both 5-HT and DA induced hyperglycaemia by triggering the release of CHH from the sinus gland of eyestalks. After injection, these two pharmacological drugs activate phosphorylase system, which causes mobilization of glycogen depots. Resultant glucose molecules leak from the tissues to haemolymph ultimately resulting in hyperglycaemia. 5-HT and DA might have activated phosphorylase system in intact prawns by inducing the release of CHH or by mimicking the action of this hormone. Conversely, these two drugs could not produce changes in haemolymph sugar levels of eyestalkless prawns. It seems most likely that 5-HT and DA exerted their effects by triggering the release of hyperglycaemic hormone from the XO-SG of eyestalks.

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Fig. 1.1: Injection of crustacean saline and different amounts of 5-HT and DA on haemolymph glucose levels in intact *M. rosenbergii*. Values, measured 90 min after injection are mean ± SD of six individual observations.

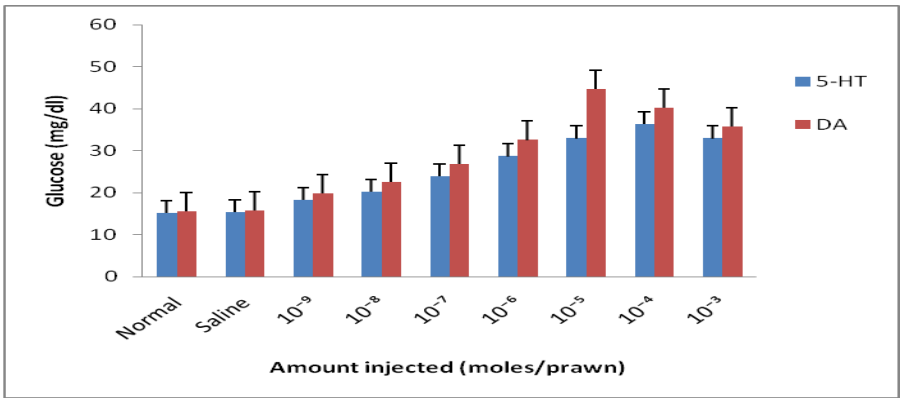

Fig. 1.2: Injection of 5-HT and DA (10^{-5} moles) on haemolymph glucose levels in *M. rosenbergii* over a period of time (min). Values are mean ± SD of six individual observations.

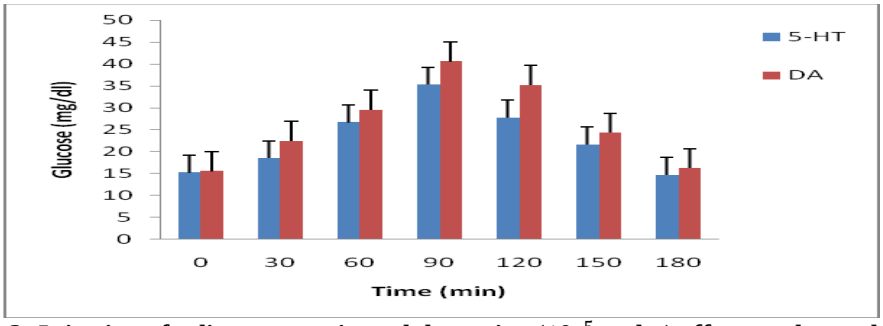

Fig. 1.3: Injection of saline, serotonin and dopamine (10^{-5} moles) effects on haemolymph glucose levels in intact and eyestalk ablated *M.rosenbergii*. Values, measured 90 min after injection are mean ± SD of six individual observations.

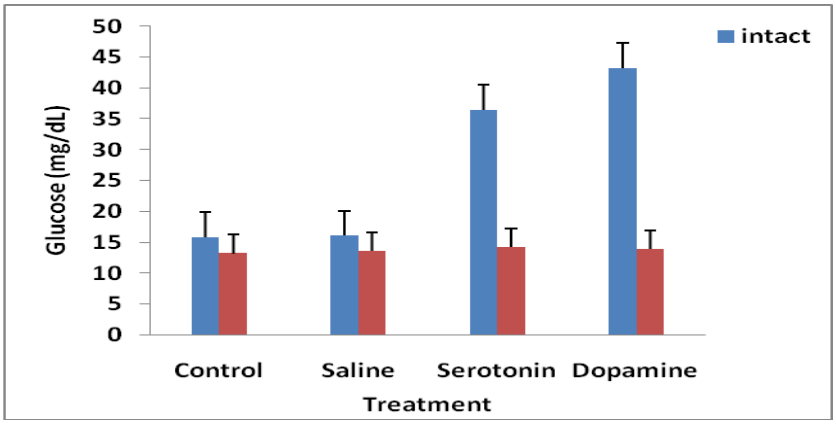

Table 1.1: Effect of injection of 5-HT and DA (10^{-5} moles) on total carbohydrate (TCHO) level (mg/gm wet weight) in the hepatopancreas and muscle of *M. rosenbergii*
Values are mean ± SD of six individual observations

Status of the prawn	Tissue	Treatment		
		Control	5-HT injected	DA injected
Intact	Hepatopancreas	15.858±0.403	11.02±0.367	10.093±0.397
Ablated	Hepatopancreas	19.356±0.601*	19.37±0.483*	19.193±0.483*
Intact	Muscle	5.298±0.289	4.326±0.172	3.963±0.082
Ablated	Muscle	7.425±0.205**	7.333±0.145**	7.186±0.126**

Values similarly marked are not significantly different (P<0.001) from each other.

Table 1.2: Effect of injection of 5-HT and DA (10^{-5} moles) on glycogen levels (mg/gm wet weight) in the hepatopancreas and muscle of *M. rosenbergii*
Values are mean ± SD of six individual observations

Status of the prawn	Tissue	Treatment		
		Control	5-HT injected	DA injected
Intact	Hepatopancreas	1.875±0.112	1.035±0.036	0.893±0.058
Ablated	Hepatopancreas	2.785±0.085*	2.513±0.076*	2.888±0.065*
Intact	Muscle	0.962±0.081	0.57±0.040	0.484±0.058
Ablated	Muscle	1.4±0.104**	1.325±0.055**	1.196±0.084**

Values similarly marked are not significantly different (P<0.001) from each other.

Table 1.3: Effect of injection of 5-HT and DA (10^{-5} moles) on phosphorylase activity in the hepatopancreas of *M. rosenbergii*
Values are mean ± SD of six individual observations

Treatment		Phosphorylase activity (µ moles of inorganic phosphate formed/mg protein/hr)		a/ab %
		A	ab	
Control (Saline injected)	Intact	3.019±0.282	5.102±0.336	59.17
	Ablated	1.894±0.174*	4.31±0.201*	43.94
5-HT Injected	Intact	3.805±0.224	6.280±0.204	60.58
	Ablated	1.669±0.182*	4.232±0.281*	39.43
DA Injected	Intact	3.941±0.226	6.348±0.221	62.08
	Ablated	1.77±0.096*	4.300±0.213*	41.16

Values similarly marked are not significantly different (P<0.001) from each other.

Table 1.4: Effect of injection of 5-HT and DA (10^{-5} moles) on phosphorylase activity in the muscle of *M. rosenbergii*
Values are mean ± SD of six individual observations.

Treatment		Phosphorylase activity (µ moles of inorganic phosphate formed/mg protein/hr)		a/ab %
		A	ab	
Control (Saline injected)	Intact	5.025 ± 0.244	7.213 ± 0.288	69.66
	Ablated	2.607 ± 0.173*	5.643 ± 0.215*	46.19
5-HT Injected	Intact	6.055 ± 0.267	8.118 ± 0.239	74.58
	Ablated	2.793 ± 0.137*	5.848 ± 0.217*	47.75
DA Injected	Intact	6.241 ± 0.237	8.256 ± 0.196	75.59
	Ablated	2.81 ± 0.171*	5.723 ± 0.215*	49.10

Values similarly marked are not significantly different (P<0.001) from each other.

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