

## Effect of 5-Hydroxytryptamine and Dopamine on the Carbohydrate Metabolism in the Shrimp, *Penaeus monodon* (Fabricius)

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**Abstract:** Effect of 5-Hydroxytryptamine (5-HT) and Dopamine (DA) on tissue carbohydrate metabolism, protein metabolism and haemolymph glucose levels were studied in the shrimp, *Penaeus monodon*. Dopamine was found to be more effective than serotonin. Serotonin and Dopamine induced hyperglycaemia only in intact prawns but not in eyestalk ablated individuals. Total carbohydrate and glycogen levels showed decrease and phosphorylase activity showed increase in the hepatopancreas and muscle of intact prawns after being injected with 5-HT/DA. However, eyestalk ablation recorded decreased haemolymph glucose and tissue phosphorylase activity and an increase in carbohydrate and glycogen levels in the hepatopancreas and muscle. At the same time total proteins, free amino acids and lipids recorded an increase in serotonin injected animals but decreased in dopamine treated animals compared to the controls.

**Key words:** *Penaeus monodon* % 5-Hydroxytryptamine (5-HT) % Dopamine (DA) % Carbohydrate Metabolism

### INTRODUCTION

The tiger shrimp, *Penaeus monodon*, is one of the most common penaeid shrimp species currently being cultured in the world. The culture of this species intensified from 1988 onwards in Taiwan and expanded rapidly to other countries in Southeast Asia. It is known that rapidly degradable environments in intensive culture ponds may result in stresses and increased incidences of disease and can lead to culture failure of shrimp crops. Stress-induced neuroendocrine changes are thought to divert an organism's energy resources away from physiological functions such as reproduction, growth and certain immune processes to metabolic and behavioral adaptations that may help the animal overcome a threat and survive [1, 2]. The effects of neuroregulators on the physiological systems of cultured shrimp are of primary concern. Several biogenic amines which function mainly as neuroregulators (i.e., neurotransmitters and neuromodulators), including serotonin, dopamine (DA), octopamine, histamine, noradrenaline (norepinephrine), adrenaline (epinephrine), tryptamine and tyramine, have been identified and quantitatively measured in crayfish, *Pacifastacus leniusculus* and other decapod crustaceans [3-5]. Among the biogenic amines, the presence of DA

and serotonin in the crustacean nervous system is well established [6]. DA is widely distributed in the crustacean nervous system and has a diverse array of physiological effects as reviewed by Tierney [7]. DA has been reported to stimulate the release of both the pigment-concentrating hormone [8, 9] and the distal retinal pigment dark-adapting hormone [10] in the fiddler crab, *Uca pugilator*, which causes the release of crustacean hyperglycemic hormone from the X organ/ sinus gland complex of *Orconectes limosus*, the shore crab, *Carcinus maenas* [11] and the freshwater giant prawn, *Macrobrachium rosenbergii* [12] and to inhibit 5-hydroxytryptamine- stimulated testicular maturation in the fiddler crab, *U. pugilator* [13] and ovarian maturation in *U. pugilator* [14], in *P. clarkia* [15] and in *M. rosenbergii* [16]. Studies on marine, freshwater and terrestrial crustaceans have indicated that DA is involved in ionic and osmotic regulation and such studies were reviewed by Morris [17]. Previous studies indicated that DA induces transient modulation of physiological responses, depresses the immune ability and increases susceptibility to *Vibrio alginolyticus* in white leg shrimp, *Litopenaeus vannamei* [18]. Li *et al.* [19] also reported that DA depresses the immune ability and increases susceptibility to *Lactococcus garvieae* in the freshwater giant prawn, *M. rosenbergii*.

In crustaceans, biogenic amines function mainly as neurotransmitters and Neuromodulators in the nervous system, with some molecules serving as circulating neurohormones. Neuroregulators are compounds that function either as neurotransmitters by acting on the transfer of information between a neuron and an adjacent target cell or as neuromodulators by amplifying or dampening neurotransmitter activity.

This paper aims at studying the effects of 5-HT and DA on haemolymph glucose levels, carbohydrate metabolism and protein metabolism of a marine prawn *Penaeus monodon* and testing the hypothesis that 5-HT and DA produce hyperglycaemia in *Penaeus monodon* by stimulating the release of hyperglycaemic hormone from XO-SG. 5-HT is a biogenic amine neurotransmitter found in both vertebrates and invertebrates that affects a wide variety of physiological and behavioral functions, including reproduction, sleep, appetite, learning, pain perception and circadian rhythm., serotonin has been shown to stimulate the release of several neurohormones such as crustacean hyperglycemic hormone, red pigment dispersing hormone neurodepressing hormone and molt- inhibiting hormone.

## MATERIALS AND METHODS

**Experimental Protocol:** *P.monodon* juveniles (14g) were obtained from a commercial farm in Muttembaka village near Nellore andhra Pradesh and were transferred to the laboratory in aerated plastic containers and maintained in laboratory holding tanks for a week in continuously aerated and filtered marine water at  $28\pm1^{\circ}\text{C}$  with a 12hr light-dark cycle. During this period the prawns were fed ad libitum with commercial pelleted feed (CP Aquaculture India Ltd., Chennai, India) once a day after changing at least 25% of the ambient medium. A constant biomass: water volume ratio (1g/1) was maintained throughout. Feeding was stopped 24hr before the start of experiments and no food was given during experimentation. The biogenic amines, 5-HT and DA, were obtained as hydrochlorides from sigma chemical Co. (St. Louis, Mo). All test solutions were prepared afresh in degassed crustacean saline [20] before the start of each experiment to avoid oxidation. The biogenic amines were tested in concentrations ranging  $10^{-5}$  mol/animal in a volume of 10 $\mu\text{l}$ . Prawns to be used in the experiments were divided into four groups, with each group consisting of six individuals. The first group received no treatment served as control. The group 2 and 3 independently received injections of 5-HT and DA ( $10^{-5}$  mol) through the base of

the cox of the second pair of walking legs respectively, in a 10 $\mu\text{l}$  volume for TCHO, glycogen and phosphorylase experiments. Both eyestalks were ablated from all the prawns in groups 4. The eyestalks were extirpated by cutting them off at the base without prior ligation with postoperative cautery of the wound. The eyestalkless prawns were used for bioassay tests two days after ablation to enable metabolism of circulating hyperglycaemic hormone. Haemolymph was collected through the arthrodial membrane at the base of a walking leg using a hypodermic syringe. The other tissues (hepatopancreas and muscle) were then quickly dissected on ice, weighed and used for further biochemical analysis.

**Haemolymph Glucose Level:** Haemolymph (100 $\mu\text{l}$ ) was mixed with 300 $\mu\text{l}$  of 95% ethanol. After deproteinization and centrifugation ( $4^{\circ}\text{C}$ , 14,000g, 10min) the supernatant was combined with a mixture of glucose enzyme reagent (glucose -6-phosphate dehydrogenase and NADP) and colour reagents phenazine methosulphate and idonitrotetrazolium chloride (loba chem., India) (procured from sigma, USA). After 30min the intensity of the colour was measured at 490nm and quantified against standards.

**Tissue TCHO and Glycogen Levels:** TCHO concentration in the hepatopancreas and muscle were estimated in 10% trichloroacetic acid (TCA) supernatant (4% w/v) and glycogen concentration was measured using the ethanolic precipitate of TCA supernatant following the method of Carroll *et al.* [21]. To 0.5 ml clear supernatant, 0.5ml of anthronine reagent was added and the contents were boiled for 10min in a water bath. The samples were immediately cooled at room temperature. A standard with a known amount of glucose was always tested along with the experimental samples. Absorbance was measured at 620nm against a reagent blank.

**Tissue Phosphorylase Activity:** Phosphorylase Activity in the hepatopancreas and muscle was estimated by colorimetric determination of inorganic phosphate released from glucose-1-phosphate (G-1-P) following the method of Cori *et al.* [22]. Tissue homogenate (5% w/v) was prepared in an aqueous medium containing 0.1M sodium fluoride and 0.037M ethylenediamine tetra acetic acid (EDTA) pH6.8 and centrifuged at 3,000 rpm for 10 min. The supernatant, diluted to four times (1:3) with cysteine hydrochloride (0.3M) sodium glycerophosphate (0.07M) buffer (pH 6.8), is used as an enzyme source. Initially 0.4 ml of enzyme was incubated with 2.0mg of glycogen for 20 min at  $35^{\circ}\text{C}$ . The reaction was then

initiated by the addition of 0.2ml of 0.016M G-1-P to one tube (phosphorylase a) and a mixture of 0.2ml G-1-P and 0.004M adenosine-5-monophosphate to an other tube (phosphorylase ab). The activity of total phosphorylase (ab) and active phosphorylase (a) was measured after incubating the reaction mixture for 15 and 30min, respectively.

**Proctien And Free Amino Acids Determination:** Total protein and free amino acid concentration in enzyme source was determined by method of Lowry *et al.* [23] using bovine serum albumin (BSA) as standard and Free amino acids were determined by Moore and Stein [24] method.

**Lipid and Fatty Acid Determination:** Total lipid was estimated by the method of Folch *et al.* [25] and Free Fatty acid content was estimated by the method of Natelson [26].

**Statistical Analysis:** A multiple comparisons (Duncan's) test was conducted to compare significant differences among treatments using the SPSS computer software and differences were considered significant when  $P < 0.001$ .

## RESULTS

Present results on the effect of injection of different concentrations of serotonin and dopamine on haemolymph glucose concentrations, Phasphorylase

activity, glycogen, Total Carbohydrates, Proteins, Free amino acids, lipids and Free fatty acids in Hepatopancreas and Muscle of *P.monodon* were studied. Injection of 5-HT and DA separately into intact prawns induced significant dose-dependent hyperglycaemia. It was also interesting to note that at this dose DA caused more hyperglycaemia than 5-HT in *P.monodon*. A time course for both 5-HT and DA-induced hyperglycaemia at  $10G^5$  mol/prawn showed clearly that haemolymph glucose levels increased significantly within 30 min of 5-HT and DA injections and reached a peak at 90 min, to decline gradually thereafter. Hence a 90 min post-injection duration was selected to study changes in carbohydrate metabolism.

Total carbohydrate(TCHO) and glycogen concentration in the hepatopancreas and muscle of 5-HTand DA injected intact prawns were significantly lower than those in the hepatopancreas and muscle of control prawns, suggesting metabolization of TCHO and glycogen and mobilization of glucose from the hepatopancreas and muscle to heamolymph. The results also showed that activity levels of phosphorylase "a" and "ab" significantly increased in both hepatopancreas and muscle following 5-HT and DA injections. The ratio of active total phosphorylase also increased in both the tissues after 5-HT and DA injections. Suggesting conversion of inactive phosphorylase to active phosphorylase. Interestingly, the magnitude of decreases in TCHO and glycogen concentrations and increase in phosphorylase (a and ab) activity levels in both the

Table 1: Effect of injection of 5- hydroxytryptamine (5- HT) and dopamine (DA) ( $10G^5$  mol) on phosphorylase activity in the hepatopancreas of *P. monodon*

		Phosphorylase activity ( $\mu$ moles of inorganic phosphate formed / mg protein / hr)		
Treatment		a	ab	a/ab (%)
Control	Intact	4.23 $\pm$ 0.05	6.62 $\pm$ 0.07	63.93
	Ablated	3.46 $\pm$ 0.01*(-18.2)	6.16 $\pm$ 0.01*(~ 13.8)	56.14
5-HT- injected	Intact	5.63 $\pm$ 0.15 (33.0)	7.72 $\pm$ 0.13 (16.6)	72.89
	Ablated	3.15 $\pm$ 0.01*(- 44.0)	6.06 $\pm$ 0.01*(- 21.5)	52.02
DA -injected	Intact	5.97 $\pm$ 0.11 (41.1)	7.89 $\pm$ 0.18 (19.1)	75.66
	Ablated	3.34 $\pm$ 0.08*(-44.0)	6.32 $\pm$ 0.11*(- 19.8)	52.80

Table 2: Effect of injection of 5- hydroxytryptamine (5- HT) and dopamine (DA) ( $10G^5$  mol) on phosphorylase activity in the muscle of *P.monodon*.

		Phosphorylase activity ( $\mu$ moles of inorganic phosphate formed / mg protein / hr)		
Treatment		a	ab	a/ab (%)
Control	Intact	6.23 $\pm$ 0.06	8.66 $\pm$ 0.01	71.81
	Ablated	4.33 $\pm$ 0.02*(- 30.4)	7.24 $\pm$ 0.07*(- 16.3)	59.65
5-HT- injected	Intact	7.67 $\pm$ 0.12 (23.1)	10.03 $\pm$ 0.16 (15.8)	76.55
	Ablated	4.97 $\pm$ 0.05*(- 35.2)	7.77 $\pm$ 0.14*(- 22.5)	64.02
DA -injected	Intact	8.03 $\pm$ 0.15 (28.8)	10.12 $\pm$ 0.17 (16.8)	79.45
	Ablated	5.12 $\pm$ 0.07* (- 36.2)	7.55 $\pm$ 0.13* (- 25.3)	67.78

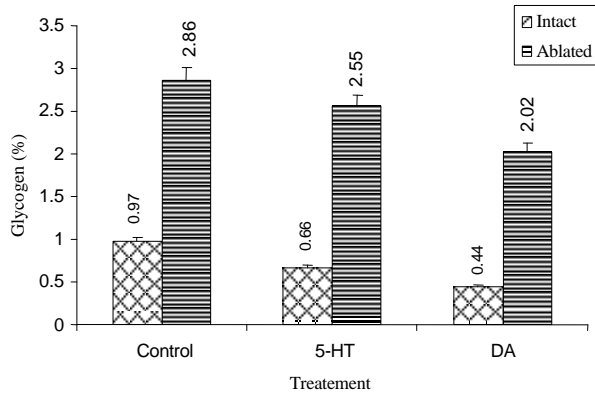


Fig. 1: Effect of injection of 5- hydroxytryptamine (5- HT) and dopamine (DA)  $10G^5$  mol on glycogen levels levels in the muscle of Intact and ablated *P. monodon*. (Values, measured mean  $\pm$  SD of six individual observations)

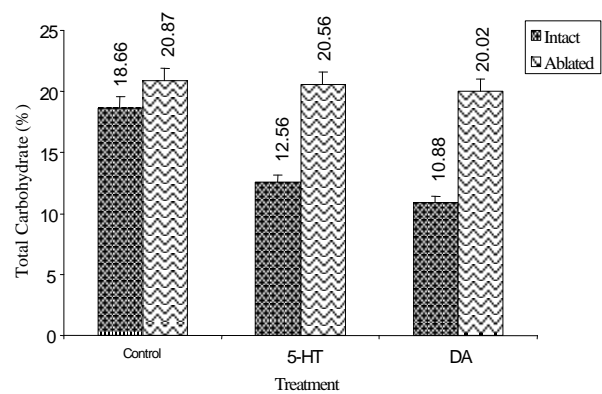


Fig. 4: Effect of injection of 5- hydroxytryptamine (5- HT) and dopamine (DA)  $10G^5$  mol on total carbohydrate(TCHO) levels in the hepatopancreas of intact and ablated *P.monodon*. (Values, measured mean  $\pm$  SD of six individual observation)

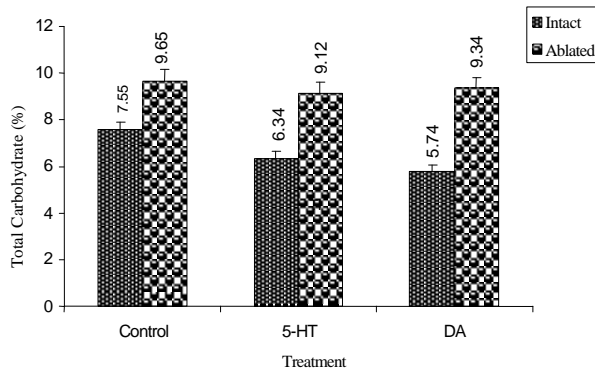


Fig. 2: Effect of injection of 5-hydroxytryptamine (5- HT) and dopamine (DA)  $10G^5$  mol on total carbohydrate (TCHO) levels in the muscle of Intact and ablated *P. monodon*. (Values, mean  $\pm$ SD of six individual observations)

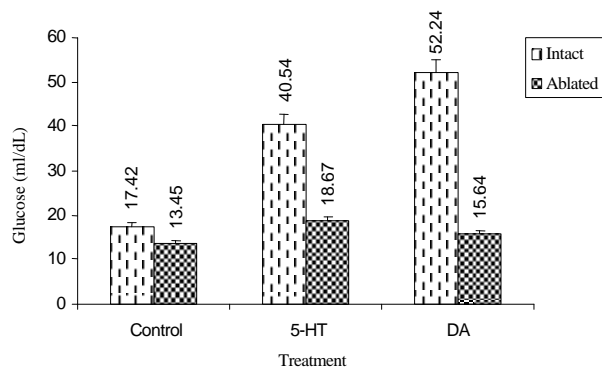


Fig. 5: Effect of injection of saline, 5-hydroxytryptamine (5-HT) and dopamine (DA)  $10G^5$  mol on haemolymph glucose concentrations of intact and eyestalk-ablated *P.monodon*. (Values are mean  $\pm$  SD of six individual observations)

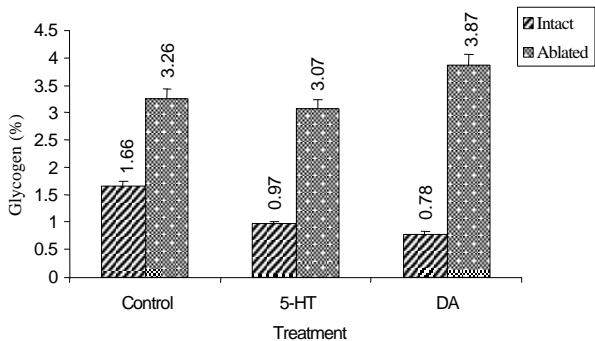


Fig. 3: Effect of injection of 5-hydroxytryptamine (5- HT) and dopamine (DA)  $10G^5$  mol on glycogen levels in the hepatopancreas of intact and ablated *P. monodon*. (Values, measured mean  $\pm$  SD of six individual observations)

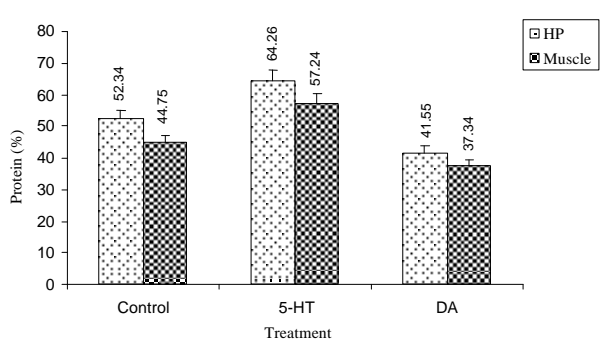


Fig. 6: Effect of injection of 5- hydroxytryptamine (5- HT) and dopamine (DA)  $10G^5$ mol on protein levels in the hepatopancreas of intact and ablated *P.monodon*. (Values are measured mean  $\pm$  SD of six individual observations)

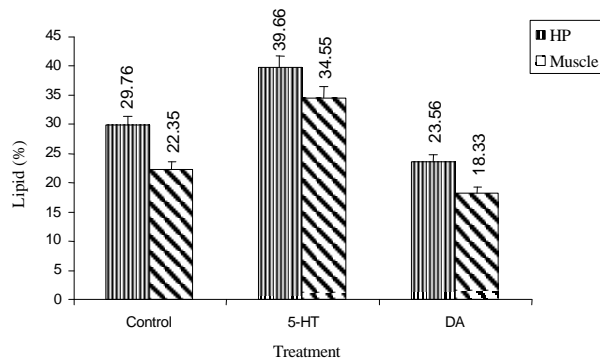


Fig. 7: Effect of injection of 5- hydroxytryptamine (5- HT) and dopamine (DA) ( $10G^5$ mol) on lipid levels in the hepatopancreas of Intact and Ablated *P.monodon*. (Values are measured mean  $\pm$  SD of six individual observations)

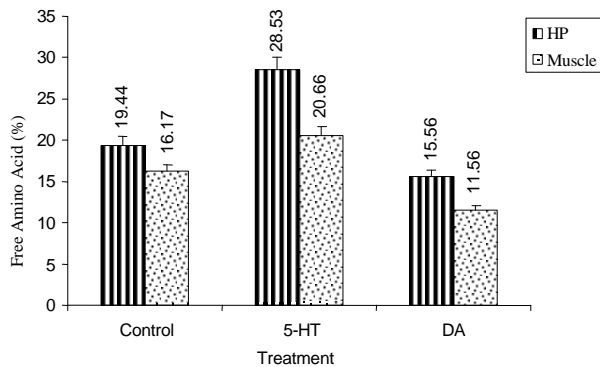


Fig. 8: Effect of injection of 5- hydroxytryptamine (5- HT) and dopamine (DA)  $10G^5$ mol on free amino acid levels in the Hepatopancreas of intact and ablated *P.monodon*. (Values are measured mean  $\pm$  SD of six individual observations)

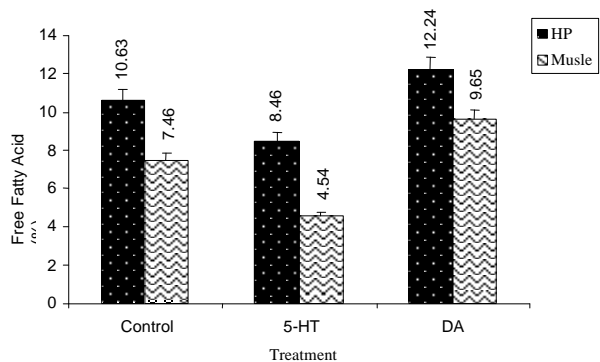


Fig. 9: Effect of injection of 5- hydroxytryptamine (5- HT) and dopamine (DA)  $10G^5$ mol on free fatty acid levels in the Hepatopancreas of intact and ablated *P.monodon*. (Values are measured mean  $\pm$  SD of six individual observations)

hepatopancreas and muscle was higher in DA injected shrimps than in 5-HT injected shrimps. Bilateral eyestalk ablation in shrimp caused a significant decrease in the activity level of phosphorylase (a and ab) in the hepatopancreas and muscle, with concomitant significant increase in tissue TCHO, glycogen concentration, proteins, free amino acids, lipids and triglycerides and a significant decrease in haemolymph glucose concentration.

The results clearly demonstrate 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 concentration and a significant decrease in haemolymph glucose concentration. Interestingly injection of both of serotonin and dopamine into eyestalk ablated prawns did not cause any significant change in the activity levels of phosphorylase in the hepatopancreas and muscle composed to eyestalkless controls.

## DISCUSSION

Stress-induced neuroendocrine changes are thought to divert an organism's energy resources away from physiological functions that do not immediately help the animal overcome a threat and survive to those that do [1]. To meet the heightened energy demands of stressed animals, glycogen, due to its easy availability for energy production, may be rapidly catabolized resulting in losses of those reserves in tissues; consequently resulting in a significant elevation in blood glucose levels. Hyperglycemia as a secondary stress response has been documented in many species of crustacean in response to a wide range of stresses [27, 12]. It is known that CHH, synthesized and released from the X-organ sinus gland complex in crustaceans, is an important neurohormone involved in glucose metabolism. Hemolymph glucose levels significantly increased in shrimp *P.monodon*, that had received dopamine and Serotonin. The effects appeared to have been dose dependent [28]. A similar trend of a hemolymph glucose increase was also observed in shrimp which had received Norepinephrine. These facts led us to hypothesize that stresses induce the release of Norepinephrine in the hemolymph and affect the target tissue in *L. vannamei*, resulting in a hyperglycemic response.

The results presented in this study suggest that 5-HT and DA are involved in the regulation of carbohydrate metabolism in the marine water shrimp, *P. monodon*. A significant increase in phosphorylase activity and decrease in total carbohydrate (TCHO) and glycogen concentration in Hepatopancreas and muscle of *P. monodon* followed by hyperglycemia indicate tissue glycogen break down and subsequent mobilization of sugar molecules from tissues to haemolymph. Similar results have been obtained in the fresh water crab, *oziotelphusa senex senex* [29, 30] and estuarine crab, *Scylla serrata* [31]. Although the eyestalk hormone that augments haemolymph glucose levels is conventionally called CHH, Hohnke and Scheer [32] suggested that the primary function of CHH was not to elevate haemolymph glucose level but to elevate intracellular glucose concentration through degradation of glycogen by activation the enzyme phosphorylase. The conversion of phosphorylase from its inactive to active form results in glycogen breakdown and the glycogen molecules thus produced might enter the haemolymph causing hyperglycaemia, a view supported by Telford [33] in crayfish. As described earlier, injection of 5-HT and DA in to intact *P.monodon* might have stimulated the phosphorylase system, causing degradation of glycogen, resulting in accumulation of sugar molecules in the tissues and concomitant release in to the haemolymph and activation of the phosphorylase system. In intact prawns injected separately with 5-HT and DA might occur either by triggering the release of hyperglycaemic hormone from XO-SG or by mimicking the action of this hormone. Apparently as 5-HT and DA did not produce these changes in eyestalkless *P.monodon*. It seems most likely that these two biogenic amines produced the hyperglycaemic hormone from the XO-SG of eyestalks.

The results that the magnitude of increase in the hepatopancreatic total lipids was more in serotonin treated prawns than in dopamine treated ones. An increase in the total lipids as a result of release of GSH by serotonin injection reflects the accumulation of vitellin during ovarian maturation. Similar results have been reported in *P.semisulcatus* for the lipid composition of purified vitellin [34]. This study reveals that serotonin treatment might have enhanced ovarian maturation as a result of increase in the hepatopancreatic lipid and free fatty acids. However, in dopamine treated prawns, inhibition of ovarian growth has been observed. This may be attributed to decrease in hepatopancreatic lipids, triglycerides, free amino acids, proteins and increase in

free fatty acid levels. A significant increase in free amino acids (FAA) content could be due to degradation of proteins. Total free amino acids (FAA) pool is very high in crustaceans and a part of this pool may be utilized to meet the energy demand [35]. Since proteins are said to be the major components of energy source in crustaceans [36], the amino acids being precursors of proteins, seem to be mobilized and converted into gluconeogenic precursors to meet extra energy requirements if needed.

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