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Induced thermal stress on serotonin levels in the blue swimmer crab, *Portunus pelagicus*

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ABSTRACT

The temperature of habitat water has a drastic influence on the behavioral, physiological and biochemical mechanisms of crustaceans. Hyperglycemia is a typical response of many aquatic animals to harmful physical and chemical environmental changes. In crustaceans increased circulating crustacean hyperglycemic hormone (CHH) and hyperglycemia are reported to occur following exposure to several environmental stress. The biogenic amine, serotonin has been found to modulate the CHH levels and oxidation of serotonin into its metabolites is catalysed by monoamine oxidase. The blue swimmer crab, *Portunus pelagicus* is a dominant intertidal species utilized throughout the indo-pacific region and is a particularly important species of Palk bay. It has high nutritional value and delicious taste and hence their requirements of capture and cultivation of this species are constantly increasing. This species experiences varying and increasing temperature levels as it resides in an higher intertidal zone of Thondi coast. The present study examines the effect of thermal stress on the levels of serotonin and crustacean hyperglycemic hormone in the hemolymph of *P. pelagicus* and analyzes the effect of the monoamine oxidase inhibitor, pargyline on serotonin and CHH level after thermal stress. The results showed increased levels of glucose, CHH and serotonin on exposure to 26 °C in control animals. Pargyline injected crabs showed highly significant increase in the levels of CHH and serotonin on every 2 °C increase or decrease in temperature. A greater CHH level of 268.86 ± 2.87 fmol/ml and a greater serotonin level of 177.69 ± 10.10 ng/ml was observed at 24 °C. This could be due to the effect of in maintaining the level of serotonin in the hemolymph and preventing its oxidation, which in turn induces hyperglycemia by releasing CHH into hemolymph. Thus, the study demonstrates the effect of thermal stress on the hemolymph metabolites studied and the role of pargyline in elevating the levels of serotonin and CHH on thermal stress in the blue swimmer crab, *P. pelagicus*.

1. Introduction

The temperature of habitat water has a drastic influence on the behavioral, physiological and biochemical mechanisms of crustaceans. Crustaceans have unique kind stress responsive mechanisms and they are well known to use that mechanisms controlled by neural and endocrine centers. Hyperglycemia is a typical reaction of crustaceans to harmful physical and chemical environmental changes. Hyperglycemia generally occurs following exposure to differing stressors, such as emersion [1], cold shock [2], anoxia and carbon dioxide [3], nitrite [4], pollutants [5] and parasitic infection [6] in crustaceans.

The release of glucose into the hemolymph is mediated by Crustacean Hyperglycaemic Hormone (CHH) through the mobilization of intracellular glycogen stores [7]. Researchers have noted that production of CHH after stressful periods during premolt is completely from the sinus gland (SG); apparently CHH from the gut is certainly not involved in the stress response [8]. Still other authors have suggested that non-SG-CHH could mediate a localized regulation of cellular glucose metabolism. Under periods of stress, localized release of CHH in the nervous system could be important in meeting the metabolic requirement of neural cells or play neuromodulatory roles in addition to endocrine functions [9]. Hence, although the contribution of this form of CHH may be insignificant to glucose regulation at the organism level, it could contribute to the regulation of the secreting organ local glucose metabolism [10].

Biogenic amines have been found to modulate their release of various neurohormones from crustacean neuroendocrine organs. Biogenic amines present in crustacean nervous systems [11,12] have been reported by Luschen et al. [13] to produce hyperglycemia in intact and eyestalk ablated shore crab, *Carcinus maenas*. The role of 5-hydroxytryptamine (5-HT/Serotonin) as an important neuromodulator of hormonal secretion has been well documented

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for several *crustacean spp*. [12]. Inhibition of the release of MIH by 5-HT has also been described by Mattson and Spaziani [14] in the crab, *Cancer antennarius. In vitro* and *in vivo* experiments carried out in the crayfish, *Procambarus clarkii*, and the crab, *Uca pugilator*, have shown that 5-HT stimulates the release of the stimulatory factor, Gonad stimulating hormone (GSH) [15]. Van Herp and Kallen [16] and Fingerman [17] have reviewed the stimulatory effect of 5-HT on CHH. Serotonin has been reported to stimulate CHH release from sinus glands of the crayfishes, *Orconectes limosus* and *Astacus leptodactylus* [18,19]. Serotonin increases the level of hemolymph glucose by inducing the release of crustacean hyperglycemic hormone in the crayfish *O. limosus* [18], in *Fenneropenaeus indicus* [20].

Direct demonstration of CHH release as a response to stressful conditions had to await methods for determination of circulating CHH in the hemolymph. Radioimmunoassays (RIAs) for CHHs of *C. maenas, O. limosus,* and *Cancer pagurus* have been developed and proved sensitive enough to measure CHH in relatively small hemolymph samples [21,22]. These assays have been used to measure CHH levels hemolymph under different experimental conditions in *O. limosus* [23], *Homarus americanus* [24] and in *C. pagurus* made hypoxic by emersion [22]. In addition, an ELISA, which proved to be of higher sensitivity than the RIA, has been developed for crab CHHs [25].

Serotonin is an amine neurotransmitter synthesized by enzymes that act on tryptophan and/or 5-hydroxytryptophan. Serotonin is stored in presynaptic vesicles and released to transmit electrochemical signals across the synapse. Serotonin is synthesized through 2-step process, involving a hydroxylation reaction (catalyzed by tryptophan-5-monooxygenase) and then a decarboxylation process catalyzed by aromatic L-amino acid decarboxylase. Serotonin is stored in presynaptic vesicles and released to transmit electrochemical signals across the synapse. Serotonin is then oxidized into 5-hydroxyindole acetaldehyde in the presence of monoamine oxidase (MAO) and its further catabolism into 5-hydroxylindole acetic acid is mediated by aldehyde dehydrogenase.

Pargyline is a monoamine oxidase inhibitor with antidepressant activity. Pargyline selectively inhibits MAO type B, an enzyme catalysing the oxidative deamination and inactivation of certain biogenic amines within the presynaptic nerve terminal. By inhibiting the metabolism of those biogenic amines in the brain, pargyline increases their concentration and binding to postsynaptic receptors. Increased receptor stimulation may cause down regulation of central receptors which my attribute to pargyline's anti-depressant effect.

Injection of pargyline has been found to inhibit the monoamine oxidase activity and elevate the presence of 5-HT content in the eyestalk and brain of *U. pugilator* [26]. Higher concentration of pargyline has been observed to result in a greater reduction of 5-HT degradation into 5-HTPH in the eyestalk of crayfish, *P. clarkii* [27]. The blue swimmer crab, *Portunus pelagicus* is a dominant intertidal species utilized throughout the indo-pacific region and is a particularly important species of Palk bay experiencing varying and increasing temperature levels. Thus a better understanding of the relationship between tolerance mechanisms against temperature in this species in laboratory condition may give rise to the possibility of more efficient control of their mortality and its higher degree of adaptability to thermal regimes. The present study examines (a) the effect of thermal shock on the levels of stress indicators i.e., hemolymph serotonin, CHH and glucose and (b) the activity of pargyline, the monoamine oxidase inhibitor, on the level of serotonin after thermal shock in the blue swimmer crab, *P. pelagicus.*

2. Material and methods

2.1. Animal collection and maintenance

Adult intermolt female crabs were procured from local fishermen of Thondi coast with the carapace length of 10 ± 1 cm and 80 ± 5 g wet weight. All the crabs were immediately transferred to the laboratory and introduced into the tank containing pre aerated filtered sea water. Crabs were acclimatized for one week in tanks containing 10–15 cm of sand at the bottom, at about 34 ± 2 ppt salinity and at a rearing temperature of 28 ± 0.5 °C and were fed with clam meat. Feeding was stopped 24 h prior to the experiment.

2.2. Heat shock treatments

The crabs were divided into four experimental groups consisting of 30 crabs each for stress treatments.

Experimental group 1: This experimental group was exposed to 24 °C.

Experimental group 2: The crabs were exposed to 26 °C.

Experimental group 3: The crabs were exposed to 30 °C.

Experimental group 4: This experimental group was exposed to 32 °C.

After 3 h of thermal stress, the experimental crabs were injected with either phosphate buffered saline or pargyline prepared in phosphate buffered saline. Groups of ten crabs each were injected with pargyline (100 mg/kg) or PBS was injected through the arthroidal membrane at the base of a swimming leg. The crabs that did not receive any injection served as control.

The crabs were then reintroduced into their ambient temperature (28 ± 0.5 °C). After a recovery period of 3 h, hemolymph was sampled from both control and experimental crabs and stored at -20 °C to access the levels of glucose, CHH and 5-HT.

2.3. Determination of hemolymph glucose levels

Determination of glucose was achieved by using the glucose oxidase method [28] in the Multiwell format. Hemolymph samples were thawed and centrifuged at 1000 rpm for 10 min at room temperature to drive Cell Free Hemolymph (CFH) from the sample. The glucose mono reagent (Span Diagnostics Ltd., India) of 1 ml was added to 10 μl CFH and was incubated for 10 min at room temperature. The sample (200 μl) was then added to well plates and read with the ELISA reader (Cyberlab Inc., USA) at 492 nm.

2.4. Quantification of hemolymph CHH and serotonin levels

HPLC-purified CHH standard from the crayfish *Orconectes limosus* (0.3–20 fmol; Fig. 1) and the anti-*Carcinus maenas*-CHH raised in rabbit were used for CHH quantification. Quantification of serotonin was carried out with serotonin creatinine sulfate standard (0.02–2500 ng/ml; Fig. 2) and the serotonin primary antibody raised in rabbit. Hemolymph CHH and 5-HT levels in the hemolymph were determined using indirect ELISA [29,30].

The cell free hemolymph samples (CFH) was mixed 1:1 v/v with coating buffer (0.2 M Sodium carbonate-bi carbonate buffer, pH-9.4) [5,22] and 100 μl was loaded in each well. The plate was incubated at 4 °C overnight. After washing with washing buffer (10 mM Phosphate Buffered Saline, pH-7.4 and 0.1% Tween 20) the plate was blocked with 100 μl of blocking buffer (10 mM PBS, 0.1% Tween 20, 2% BSA) for two hours at room temperature. After washing, the wells were incubated with anti-*Carcinus maenas*-CHH or anti-serotonin antibody (Sigma, S5545) (dilution 1:10000 in blocking buffer) for 2 h at room temperature. Wells were then washed and incubated with the secondary antibody, Anti-Rabbit