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Elevation of Glucose on the Ablation of Single Eyestalk of the Giant Fresh Water Prawn, *Macrobrachium rosenbergii*

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Abstract

Eyestalk ablation of the giant fresh water prawn, *Macrobrachium rosenbergii* resulted in precocious moulting and an increase in the concentration of glucose, total protein and total free amino acid in its haemolymph. Alanine and aspartate aminotransferase activities also showed an increase after eyestalk ablation along with an increase in glucose level in its haemolymph. The results demonstrate precocious moulting of the prawn in response to eyestalk abaltion is associated with an elevated titre of glucose and aminotransferases activities in its hamolymph indicating gluconeogenesis during that period.

Keywords: Eyestalk ablation, *Macrobrachium rosenbergii*, aminotransferases, gluconeogenesis

Introduction

The endocrine system of decapod crustaceans consists of epithelial-type endocrine glands and endocrine structures of neural origin, the neurosecretory cells and neurohaemal organs. The X-organ/sinus gland complex is the major endocrine and control centre located in each eyestalk of most stalk-eyed crustaceans. The sinus gland, a neurohaemal organ is a storage and release center for several hormones produced by the X-organ. These hormones include moult-inhibiting hormone (MIH), crustacean hyperglycemic hormone (CHH), gonad inhibiting hormone (GIH) and several pigmentary effector hormones (7) and (14). The following functions are claimed for hormones released by the tissue- moult inhibition, control of sugar metabolism, expression of gonad development and control of pigment dispersion (17).

In hatcheries and prawn farms, the technique of eyestalk ablation has long been employed to induce female maturation; the basis of this is the removal of the eyestalk and thus the removal of the source of these inhibitory hormones.

Material and Methods

The giant fresh water prawn, *Macrobrachium rosenbergii* were collected and maintained in the laboratory in separate tanks. The prawns were grouped into two: one group with one of the eyestalk removed and the other group as normal control and were kept in separate tanks. The biochemical analyses were conducted with the haemolymph of eyestalk ablated and non-ablated prawns.

Glucose was estimated according to the method of Nelson (13) and Somogyi (18). The enzymatic activities of alanine and aspartate aminotrasferases were estimated according to the method of Reitman and Frankel (15). One unit of enzyme activity was defined as micromole pyruvate formed per minute at 37^{o}C. The specific activity of the enzyme was calculated on the basis of micromole pyruvate formed per minute at 37^{o}C per gram crude protein in the homogenate. The total proteins were estimated according to the method of Lowry et al. (10). Bovine serum albumin (Fraction V, Sigma) was used as standard protein. The total free amino acid was estimated according to the method of Lee and Takahashi (9).

Results

The biochemical components, namely, glucose, alanine and aspartate aminotransferase activity, total protein and total free amino acid were assayed in the haemolymh of eyestalk ablated and normal prawn. The removal of eyestalk resulted in precocious moulting in *Macrobrachium rosenbergii*. Moulting of prawns was marked between 10th and 15th day of experiment. All the experimental animals entered premoult within 5 days after the eyestalk removal.

Glucose

The changes in the concentration of glucose in the haemolymph showed that in the eyestalk ablated prawn the glucose titer was higher than the normal. The glucose content was initially low followed by a gradual increase on the 6th day which marks the premoult stage (Figure 1). On the 10th day the glucose titer decreased but was still higher than the normal. The glucose concentration was found to show a gradual decline in subsequent days.

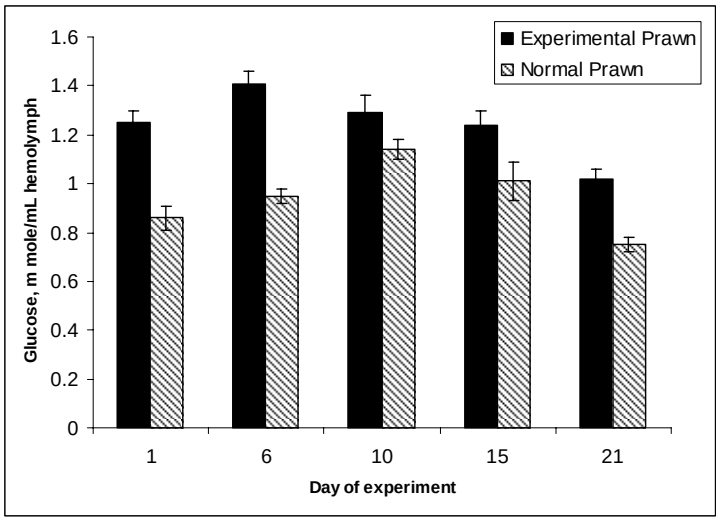

Figure 1: Changes in the concentration of glucose in the haemolymph of the eyestalk ablated and normal prawn

Alanine and aspartate aminotransferase activity

The alanine and aspartate aminotransferase activity was found to be higher in the experimental prawn when compared to the normal on the 1st day of experiment. Then it declined gradually followed by a gradual increase on the 10th day of experiment. In the eyestalk ablated prawn, the peak was observed on the 10th day followed by a decline thereafter (Figure 2 and 3). The specific activity of alanine aminotransferase and aspartate aminotransferase was found to be higher in the eyestalk ablated prawn when compared with normal.

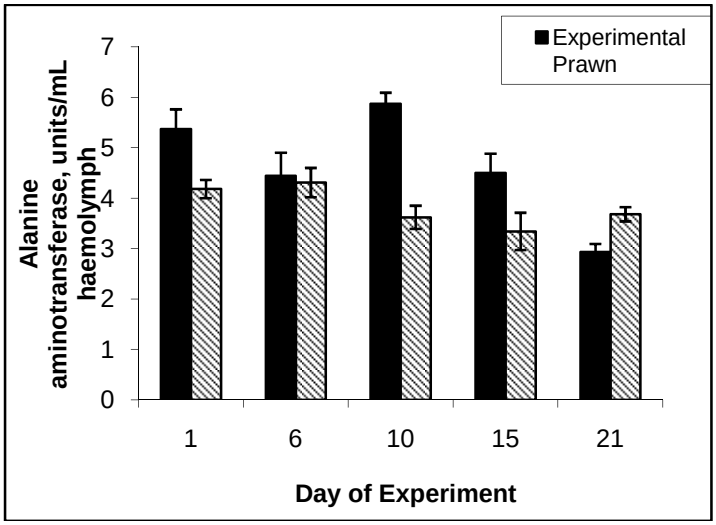

Figure 2: Changes in the alanine aminotransferase activity in the haemolymph of the eyestalk ablated and normal prawn.

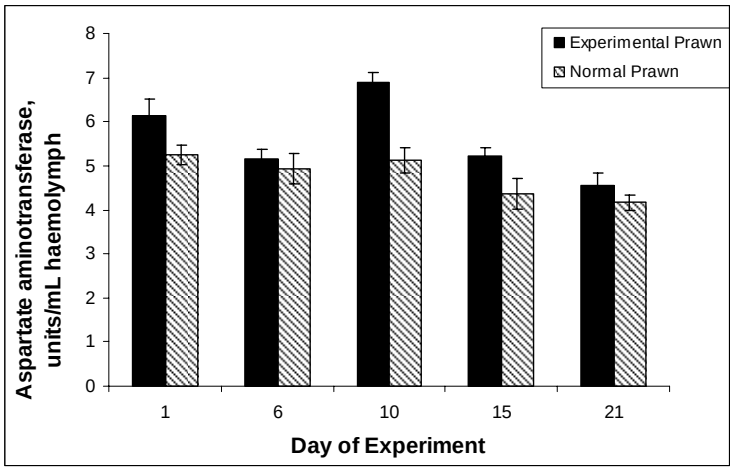

Figure 3: Changes in the aspartate aminotransferase activity in the haemolymph of the eyestalk ablated and normal prawn.

Total Protein

The total haemolymph protein in the eyestalk ablated prawn was found to be greater than the normal. In the haemolymph of eyestalk ablated prawn, the total protein content steadily increased with development and reached a peak followed by a gradual decline in the total protein concentration in the remaining days (Figure 4).

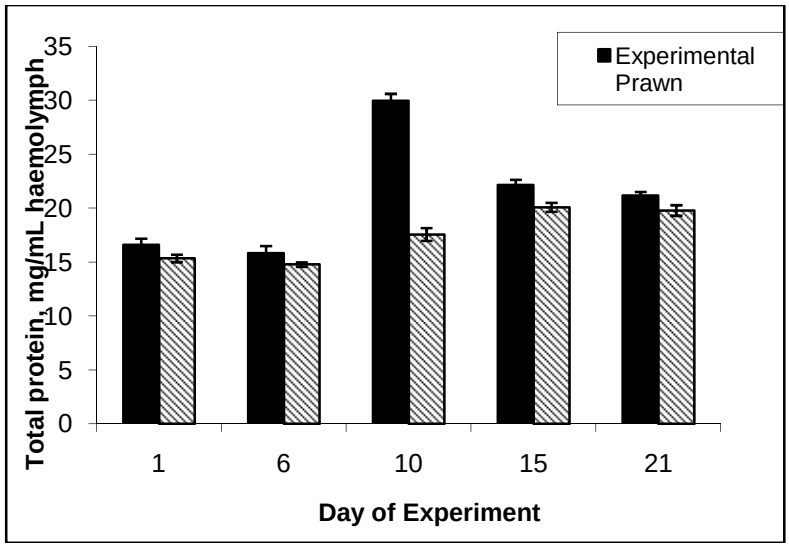

Figure 4: Changes in the concentration of total protein in the haemolymph of the eyestalk ablated and normal prawn.

Total free amino acids

The total free amino acid concentration in the haemolymph of the eyestalk ablated prawn was higher when compared to that of non-ablated prawn. In the experiment prawn, the concentration of total free amino acids increased on the 1st day of

experiment. It then declined gradually with a rapid elevation on the 10th day (Figure 5). This was followed by a decline in the concentration of total free amino acids in the subsequent days.

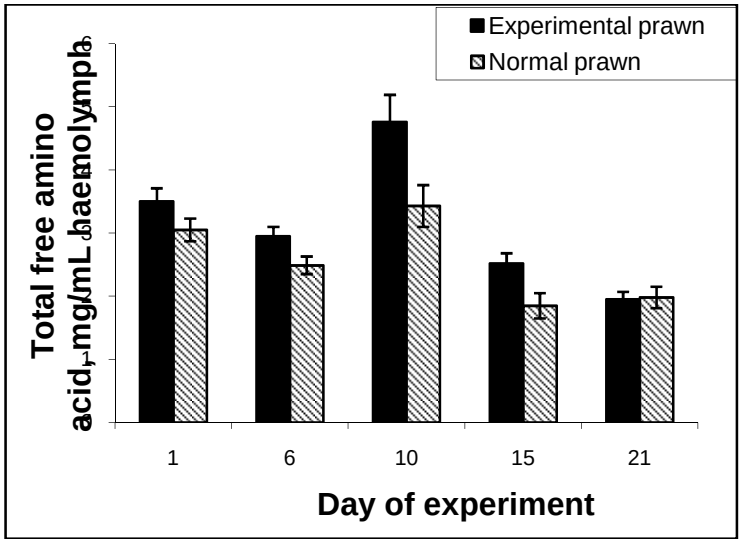

Figure 5: Changes in the concentration of total free amino acid in the haemolymph of the eyestalk ablated and normal prawn.

Discussion

Eyestalk ablation removes the X-organ and thereby releases the Y-organ from inhibition and thus initiates processes leading to moulting (5). The removal of eyestalk may result in the abrogation of MIH leading to the secretion of moulting hormone by the Y-organ which in turn accelerated the moulting process in the prawn.

CHH plays a central role in metabolic processes, especially in the regulation of blood sugar level, and is also involved in the control of reproduction, moulting and osmoregulation. An increase in glucose level was observed in the eyestalk ablated prawn. Hohnke and Scheer (4) suggested that the function of CHH is to raise the glucose level. But in the present study it was found that the glucose level rose in the eyestalk ablated prawn when the results were compared with normal. Chang et al. (1) observed that eyestalk ablated lobsters continued to produce CHH, even though the only source of CHH had been removed. Marked elevation of haemolymph glucose levels were observed in the crab, *Potamon persicum* after the removal of eyestalks. Khazraeenia and Khazraiinia (6) suggested that there are other sites distinct from the eyestalks that are sources of CHH. An increase in glucose level was seen during premoult and the source of this hormone was not from eyestalk neurosecretory tissue but from endocrine cells in defined areas of foregut and hindgut (2, 21). The result of the present study is in agreement with the above.

Aminotransferase activity has been studied in relation to the larval metabolism of insects under normal and experimental conditions (3, 12, 16). It has been shown that the level of aminotransferase increases with the growth of larva, declines during the

pupal stage and rises during the adult development (8). In *Macrobrachium rosenbergii*, both alanine and aspartate aminotransferase activities showed changes during the development of prawn. The activity of both showed an increase after moulting, thereafter, the activity showed a gradual decline. This hike is attributed to the fact that with growth, the aminotransferase activity also increases. During this period, the level of glucose was also found to be higher indicating an elevated level of gluconeogenesis during this period.

In the study an increase in the total protein content was observed in the eyestalk ablated prawn. This observation is consistent with earlier findings of Varadaraj (20). There is generally an increase in protein concentration before moult followed by a sharp fall immediately after ecdysis (19). This is due to the absorption of water and the consecutive dilution of blood. It was observed that the total free amino acid content was higher in the ablated prawn than the normal. The increase in the total free amino acid coincides with an increased rate of protein synthesis (11).

Conclusions

The eyestalk ablation resulted in precocious moulting and faster growth in *M. rosenbergii*. Removal of eyestalk resulted in an increase in the alanine and aspartate aminotransferase activities in the haemolymph of the eyestalk ablated prawn. The glucose titer in the haemolymph of the eyestalk ablated prawn was found to be higher in the premoult stage and lower in the postmoult. In general there is an increase in the concentration of total protein during moult followed by sharp fall immediately after ecdysis. The level of total free amino acids showed an increase in eyestalk ablated prawns when compared to that of control.

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