NEUROENDOCRINE REGULATION OF TISSUE AMYLASE ACTIVITY IN THE CRAB, *BARYTELPHUSA GUERINI*

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Hepatopancreatic enzyme, amylase is the most important starch splitting enzyme. Its synthesis is influenced by eyestalk hormones. The neurosecretory system in the eyestalk of *Procambarus clarkii* is involved in the regulation of digestive enzymes of hepatopancreas. Synthesis of amylase by hepatopancreas is greatly reduced when eyestalks are removed (Fingerman et al., 1967). Similar observation was also made in the freshwater prawn *Caridina weberi*, where the bilateral eyestalk ablation led to decreased amylase activity of digestive juices and hepatopancreas (Jyothi & Nagabhushanam, 1977). In the present study, an attempt was made to study the influence of eyestalk hormones on amylase enzyme activity in a freshwater crab species at different periods of eyestalk ablation and its injection.

Freshwater Crabs, *Barytelphusa guerini* (Milne Edwards) were collected from paddy fields. They were fed with slices of frog muscles and acclimatized to laboratory condition for a week. Only healthy and active animals with intact appendages were used for experimentation. Bilateral eyestalk extirpation was made by making a deep incision at their bases with a sharp blade, and the animals remained airtight with no apparent adverse effect. For preparation of eyestalk extract, enough number of eyestalks were taken and the external hard exoskeletal parts and retinal portions were removed. The internal soft portions containing the sinus gland and x-organ complex were triturated into a fine paste in a glass homogeniser and suspended in enough quantity of distilled water to make a final concentration of a pair of eyestalks / 0.2 ml. The extract was centrifuged for 10 minutes at 3000 rpm and the clear supernatant was adjusted to the final volume. 0.2 ml of this aqueous extract, which is equal to implanting a pair of eyestalks, was used for injection purposes.

The ablated animals were used for analysis after 1,4,7,10 and 15 hr of ablation; remaining animals were injected with eyestalk extract and were used for analysis after 1.4.7.10 and 15 hr of injection. Side by side normal animals with intact eyestalks served as control.

Foregut tissue and hepatopancreas were homogenised separately in phospate buffer (pH 7.4) so as to get 1% homogenate and the amylase activity was estimated in normal, eyestalk ablated and eyestalk extract injected animals. This activity (1,4 - Gluco maltodehydrolase E.C.3.2.1.1) was determined using the method of Bernfeld (1955) and using alkaline 3:5 dinitrosalycilic acid (DNS) reagent to determine the concentration of reducing substances produced in terms of maltose. The enzyme activity was expressed as mM maltose/gm wet wt / hr.

A minimum of six observations were taken in each case and the data were expressed as mean $\pm$ SD. Percent change in value was calculated by comparing the value in ablated and injected animals with the value of normal animals obtained on the corresponding day. Student 't' test was performed by comparing the values obtained in ablated and injected animals with normal animals, for determining the significance of change keeping the level of confidence at $P < 0.05$.

The results show that amylase activity in the hepatopancreas on eyestalk ablated animals decreased by 25.5% - 37.7%, during the course of experimentation over the normals; it was significant ($P<0.05$ to $P < 0.01$). On injection of eyestalk extraction the enzyme activity was restored to near normal level and the observed differences in the enzyme activity between normal and extract injected animals were not significant.

Similarly the enzyme activity in the digestive juices of the normal animals also decreased on eyestalk ablation by 13.04%, over the normal, on the Ist day, to 42.72% decrease by 15th day. This decrease was significant in all the days. Eyestalk extract injection prevented this decrease and the values obtained in the injected animals were not significantly different from those of the normal animals.

The results presented above clearly indicate that the eyestalk hormone controls the hepatopancreas functioning in the freshwater crab, *B. guerini*. While the changes observed in the normal animals experienced over the experimental period, those observed in ablated and extract injected animals are evidently due to the involvement of eyestalk hormone, in addition to the starvation effect. The eyestalk ablation leads to changes in hepatopancreas function and injection of eyestalk extracts into ablated animals reverses these changes, suggests the involvement of neurosecretory principle from x organ - sinus gland complex of the eyestalk in the control of hepatopancreas function.

Degenerative changes in the hepatopancreas on eyestalk removal and retardation of such degeneration by implantation of sinus glands into eyestalkless animals is already reported in *Procambarus clarkii* (Yamamoto, 1953). Fingerman et al. (1967) reported that eyestalk hormone is involved in regulating the enzymes of the hepatopancreas in *P. clarkii*. They reported that the amylase activity of

hepatopancreas increases in normal animals with time while in ablated animals it is less and steady. The enzyme activity in the digestive juices, on the other hand, increases in the normals but declines in the ablated animals with experimental period. Similar observations were made in *Caridina weberi* (Jyothi & Nagabhushanam, 1977) and *Barytelphusa cunicularis* (Nagabhushanam & Diwan, 1974).

In present studies, increased amylase activity in the hepatopancreas of the normal crabs may be due to the fact that they continue to synthesize the enzyme inspite of their being starved. Since they are not being fed, there is no requirement to release this enzyme and consequently it is stored in the hepatopancreas, which accounts for the observed increase in the enzyme activity in the normal crabs.

Significant reduction in amylase activity of hepatopancreas in ablated animals is due to the loss of eyestalk hormone and consequent inability to synthesize the enzyme. Bilateral eyestalk ablation impairs the enzyme synthesizing ability which accounts for the decreased enzyme activity in the hepatopancreas and the digestive juice. Restoration of the enzyme activity to the normal levels on eyestalk extract injection is due to the restoration of enzyme synthesizing capacity due to the presence of the eyestalk factor. Thus, the eyestalk factor regulates the production and secretion of digestive enzyme amylase by the hepatopancreas.

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Chromosome 22 deciphered

An international team of scientists reported that it has deciphered the genetic code of an entire human chromosome for the first time – a major milestone to decode in detail all of the chromosomes that make up the human genome.

Publishing their results in the recent issue of the scientific journal *Nature,* researchers from England, Japan and the United States say they have completed the decoding work on chromosome 22, the second-smallest of the human chromosomes, but still a sizable volume in what has been called the encyclopedia of human life.

The payoffs for decoding the human chromosomes are likely to be enormous, in predicting, diagnosing and treating human ailments such as cancer, arthritis and heart disease – and perhaps in being able to forestall the effects of aging.

Chromosome 22 contains genes thought to play a role in immunity, heart disease, schizophrenia, mental retardation and several cancers, including a form of leukemia. Knowing the sequence will almost certainly lead to a deep understanding of what goes awry in these diseases–and should help biotech and pharmaceutical companies in developing medications to treat them.

The scientists painstakingly determined the order of the *33 million* chemical building blocks linked together in a long molecule of DNA that runs through the chromosome. Printing out the exact sequence of these compounds in chromosome 22, using the letters A,T,C or G to stand for the chemicals adenine, thymine, cytosine and guanine, would fill a very large book.

The scientists make clear that this new information, covering just one of the 23 pairs of chromosomes, at the center of virtually every human cell, is just a beginning – an important step in a decade long effort funded by the USA and other governments and Great Britain's Welcome Trust. But there is still a sense of awe surrounding the accomplishment. "To see the entire sequence of a human chromosome for the first time is like seeing an ocean liner emerge out of the fog, when all you've ever seen before were rowboats," said Dr Francis Collins, Director of the National Human Genome Research Institute.

Despite the congratulatory air, the researchers are aware that they are in competition with private companies anxious to claim the territory as their own. In a commentary, one scientist wrote that "it would be a terrible blow for science and humanity if the human genome became a commercial property." To avoid that from happening, scientists in the international consortium have agreed to make their work freely available on the Internet within 24 hours of completing the sequence for each small piece of DNA.

There are still 11 gaps along the chromosome, which may take another 10 years' work.

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