Influence of calcium on corpuscles of Stannius activity in the freshwater fish, *Notopterus notopterus*

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Abstract

The Corpuscle of Stannius (CS) cells were not active under distilled water exposure. The CS cells have small nucleus and intense eosinophilic cytoplasm without any vascularisation indicating that the contents are not released in the CS cells. In calcium chloride exposed and injected fish, the CS cells are active characterized by a vesicular nucleus in the majority of cells with cytoplasmic degranulation. The high protein content was observed in the corpuscles of Stannius (CS) in the fish exposed to distilled water. In calcium chloride injected fish, the protein content of CS found to be decreased in comparison to distilled water exposure and control indicating release of protein contents from the CS cells. Thus the results from the above studies suggests that calcium content of the medium plays significant role in stimulating and activating corpuscles of Stannius cells in the freshwater fish, *Notopterus notopterus*.

Keywords: Calcium, corpuscles of Stannius, distilled water, *Notopterus notopterus* and nucleus

Introduction

The degree of histological stimulation of corpuscles of Stannius depends upon the presence of calcium in the external medium. According to the experiment carried out in normal and calcium deficient sea water (Cohen et al., 1975) and in calcium enriched or calcium dependent freshwater (Wendelaar Banga et al., 1976), the corpuscles of Stannius activity appears to be regulated rather by the calcium content of the external medium than by the total osmotic concentration. Since calcium is a factor for stimulation of corpuscles Stannius in the present study, the response of corpuscles of Stannius in the freshwater fish, *Notopterus notopterus* was studied by exposing the fish to calcium deficient water i.e., exposing to distilled water and also exposing the fish to calcium rich water and injected with calcium solution.

Copper is present in natural waters as a result of industrial processes and other anthropogenic contamination of copper in excess known to cause severe toxic effects on aquatic organisms. Although copper toxicity and its effect on aquatic organism including fish has been studied, the effect of copper as a contaminant on the corpuscles of Stannius of fish under exposure has not been reported. Hence, in the present study, the effect of copper sulfate exposure on the responses of corpuscles of Stannius in the freshwater fish, *Notopterus notopterus* has been studied.
Materials and Methods

Around 70 fish *Notopterus notopterus* were utilised for the following exposural studies collected from Bheema river near Gulbarga. Ten fish were exposed to distilled water in a separate aquarium tank for 5 days and ten fish were exposed to calcium chloride solution at a concentration of 0.1% for 5 days. Ten fish were injected with calcium solution (calcium Sandoz-10% (10 ml) Korten Pharmaceuticals Pvt. Ltd.), at a dose of 0.05 ml / fish for 5 days.

All the above exposed fishes were sacrificed after termination of the experiments and the corpuscles of Stannius were dissected out and fixed in Bouin’s fluid for further dehydration, embedding in paraffin wax, sections were stained with haematoxylin and eosin. For distilled water and injected fish the CS was processed for protein estimation. The protein estimation was carried out by applying the method of Lowry *et al.* (1951). The stained CS sections were observed initially under light microscope and the changes of CS cells were recorded and photographed using OLYMPUS Binocular Microscope DP-12, Olympus BX 51, Model-ULH 100HG, Olympus Optical Co. Ltd. Made in Japan.

Results

The corpuscles of stannius (CS) cells under distilled water exposure seems to be not active. The CS cells exhibit small nucleus and intense eosinophilic cytoplasm without any vascularisation indicating that the contents are not released in the CS cells (Fig. 1). In calcium chloride exposed and injected fish, the CS exhibits the active condition characterised by vesicular nucleus in the majority of cells with cytoplasmic degranulation (Fig.1).

The protein content of the corpuscles of Stannius (CS) estimated indicate that there is a high protein content in fishes exposed to distilled water than the control ones. In calcium chloride injected fish, the protein content of CS found to be decreased in comparison to distilled water exposure and control indicating release of protein contents from the CS cells (Table – 1 and Fig. 2).

Discussion

Hanssen *et al.* (1992) demonstrated in the eel, *Anguilla anguilla* that when sea water acclimated eels were transferred to freshwater or distilled water, there appeared to be a very rapid reduction in the secretary activity in the corpuscles of Stannius as indicated by rapid accumulation of secretary granules suggesting that the secretary activity of the corpuscles of Stannius is rapidly reduced when a hypercalcimic challenge is removed. Since the corpuscles of Stannius activity depends on the calcium concentration of the medium, in the present study after exposing fish, *N. notopterus* to distilled water the corpuscles of Stannius became inactive indicating inactivation because of absence of calcium in the medium. It is known that steniacalcin hormone of the corpuscles of Stannius involved in the absorption of calcium through gills and intestine of the fish (Lafeber and Perry, 1988; Lefebre *et al.*, 1988).

The hypercalcemic factors have been the subject of less study. In some experimental studies it has been demonstrated that the hypercalcemic condition can be induced in fish (Fleming *et al.*, 1964; Fontaine *et al.*, 1964; Oguri as Tikada, 1967). Hypocalcemia is accelerated in deionised water in the eel (Olivereau and Olivier, 1978 and Charter Jones *et al.*, 1966). In the present study, when the freshwater fish, *N. notopterus* were exposed to calcium rich water and also injected with calcium solution, the corpuscles of Stannius become active indicating the release of the hormone for calcium homeostasis. This is further supported by the estimation protein content of the gland.
that the level of protein is reduced in calcium injected or exposed fish. The increase in CS protein after distilled water exposure reflects storage of the hormone stanniocalcin in the CS and reduction after calcium exposure or administration reflects the release of the hormone, suggesting that calcium is a factor for hormone release from the CS in the freshwater fish, *N. notopterus*. A relationship between the activity of the CS and the calcium concentration of the water has been suggested (Aida et al., 1980; Wagner et al., 1989). In the fish, *Tilapia* long term elevation of the calcium concentration of the water led to an increase in CS volume accompanied by an elevation of the plasma calcium concentration (Urasa and Wandelaar Bonga, 1987). Further, such relationship has been also

Fig. 1a. Showing section of corpuscles of Stannius in the control fish, *Notopterus notopterus* H&E X 1200

Fig. 1b. Showing section of corpuscles of Stannius in the distilled water exposed fish, *Notopterus notopterus* H&E X 1200

Fig. 1c. Showing section of the corpuscles of Stannius in calcium rich water exposed fish, *Notopterus notopterus* H&E X1200

Fig. 1d. Showing section of corpuscles of Stannius in the calcium injected fish, *Notopterus notopterus* H&E X 1200

Table 1. Showing Protein content of corpuscles of Stannius of freshwater fish, *Notopterus notopterus*

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Samples</th>
<th>Concentration of Protein (µg / mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>64 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>Distilled water</td>
<td>83 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>CaCl₂ injected</td>
<td>18 ± 0.10</td>
</tr>
</tbody>
</table>

Fig. 2. Showing Protein content of corpuscles of Stannius of freshwater fish, *Notopterus notopterus*
demonstrated in vitro studies by (Aida et al., 1980) and (Wagner et al., 1989) that increased medium calcium concentrations enhanced the depletion of CS secretory granules and the release of stennocalcin hormone (STC). Such observation is also made in vitro studies on the CS of eel showed that STC altered by an increase in extracellular calcium concentration well above that shown to follow most hypercalcic challenges (Hanssen et al., 1991). Flik et al. (1989a) have shown that hypocalcaemia (induced by CaCl₂ injection or sea water exposure of the fish) reduced the hypocalcin content of CS in trout, goldfish and eel; concomitantly the synthetic activity of CS of hypercalcemic fish, as determined in vitro was enhanced.

References


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