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Responce of Corpuscles of Stannius in the Freshwater Fish Notopterus Notopterus Exposed to Deferent Calcium Concentration

Pruthvi raj C.Bedjargi — Fish Endocrinology Research Laboratory, Department of Studies in Zoology, Gulbarga University, Gulbarga, Karnataka INDIA-585 106

R.S.Kulkarni — Fish Endocrinology Research Laboratory, Department of Studies in Zoology, Gulbarga University, Gulbarga, Karnataka INDIA-585 106

ABSTRACT

The involvement of corpuscles of Stannius (CS) of the freshwater fish Notopterus notopterus, in serum calcium level changes has been investigated. The changes in the serum calcium level and histology of CS cells have been studied based on exposing fish to calcium concentration and CS extract administration. The results indicate that on exposure to calcium rich water for 15 days, the serum calcium increases and after 15 days the level decreases. The CS extract injected to fish exposed to calcium rich water also exhibited initial increase of serum calcium and gradually decreases upto 15 days, The CS cells under distilled water exposure are 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. In calcium chloride injected fish, the CS cells exhibits active condition characterised by vesicular nucleus in the majority of cells with cytoplasmic degranulation suggesting that calcium is a factor for stimulation of CS for its secretion in regulating serum calcium level in the freshwater fish, Notopterus notopterus. The changes in the histology of CS cells were also noticed in the above experimental conditions.

KEYWORDS Notopterus notopterus, Corpuscles of Stannius, Calcium level, CS extract.

Introduction:

The corpuscles of Stannius (CS) have been most consistently implicated in the control of plasma calcium metabolism (Wendelaar Bonga and Pang, 1991). Stanniocalcin (STC), the hormone secreted by the CS, lowers plasma calcium levels by reducing gill calcium uptake (Fenwick and So, 1974; So and Fenwick, 1979; Milet etal. 1979; Lafeber and Perry, 1988; Lafeber et al., 1988a). Histological examination of the CS has revealed that the glands are consistently more active in sea-water-adapted fish than in freshwater adapted fish (Wendelaar Bonga et al., 1976). In addition, CS of fish kept in calcium deficient sea water appear to be inactive (Cohen et al., 1975; Wendelaar Bonga et al., 1980), suggesting that glandular activity is directly related to environmental calcium concentrations.

The corpuscles of Stannius were recognized as endocrine glands of a specific nature of the two main functions attributed to the CS are control of calcium metabolism and cardio vascular action. The CS are small ovoid endocrine glands unique to teleostean and holostean fishes embryologically, the CS may arise from the pronephric. mesonephric or opisthonephric ducts (Belsare 1973; Krishnamurthy 1976). The corpuscles of Stannius (CS) are enigmatic glands which vary in number and are usually embedded in the kidneys 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 (Wandelaar 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 activity of corpuscles of Stannius in the present study. The response of the 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.

Since role of CS in fish has not been still established. However, there are some reports that CS secretes a glycoprotein hormone which regulates calcium homeostasis . (Lafeber et al., 1988 Agber and Rebfro, 1994). Hence, in the present investigation effect of CS extract on calcium regulation has been studied in the freshwater fish Notopterus notopterus.

Materials and Methods:

The fish, Notopterus notopterus, weighing 90-100 ± 10 gms were collected from Khaja Kotanoor reservoir 15 km away from Gulbarga University campus. The live fish were brought to the laboratory and kept in large plastic tanks having size of 90cms in diameter and 60cms in height for acclimatization to the laboratory conditions for a week. Fishes were fed with live earth warms and boiled egg albumen. The unused food was removed after 2 hours and water was changed daily. Around 200 fishes of same weight were selected and divided in to three equal groups. All were sexually mature, between two to three years of age and appeared in healthy and vigorous condition.The study was conducted during the month of January 2006. The first(I) group fishes maintained in natural water as control. Second (II) group fish were maintained in calcium rich water (0.1% $CaCl_2$) solution both group-I & II injected with (0.5 ml/ 100 gm bw) 0.6 mM NaCl solution and the third group fishes were maintained in calcium rich water (0.1% $CaCl_2$) and 0.5 ml CS extract injected .

Preparation of the CS extract:

CS tissue was collected from mature fish and homogenized in a morten with appropriate volume of saline solution. Homogenate was centrifuged at 3,000 rpm for 10 min and the supernatant was filtered and used.

All the experimental and control fishes were netted from the water and blood was taken via a syringe inserted in to the intra peritoneal .The blood sampling was completed within a period of 2 minuets and by using the modified method (A.C.Ded 2002) was employed for estimating the calcium in the serum, the result obtained being mg. Ca per 100 ml serum.

The fish were killed by decapitation. In each group of three or more animals the body cavity was opened and the kidney removed. A standardized region of the musculature [Full flank dorsal to the lateral line] was removed from the fish and all the tissues fixed in a fixative Bouin's fluid containing Picric acid,Formalin&Glacial acetic acid. About an hour later the kidney, containing the corpuscles of Stannius, were dissected out and returned to fresh fluid. This procedure insured rapid fixation of the rather labile granules of the glandular tissue. followed by dehydration in graded alcohols and embedded in paraffin blocks using standard techniques, five to six micron thick sections were cut, dried and dehydrated for Harri's Hematoxylin and eosin stain. Microphotographs were taken using Labophot-2 Olympus magnifier microscope with a Nikon digital camera.

Exposural Studies:
The remaining 70 fishes were utilised for the following exposural studies.

1) Ten fish were exposed to distilled water in a separate aquarium tank for 5 days.
2) Ten fish were exposed to calcium chloride solution at a concentration of 0.1% for 5 days.
3) 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.
4) Ten fishes were exposed to copper sulfate at a concentration of 30 ppm for 4 days.Before exposing the fish to the concentration of Cupper sulfate at 30ppm, the mortality of the fish was tested under different concentration of Copper sulfate and the LC-50 i.e., 50% of mortality was observed at 30ppm and the LC-50 concentration were taken as concentration for exposural for studies for 96 hrs.

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, section preparation and staining. For distilled water and calcium chloride exposed and injected fish, the CS was processed for protein estimation.

Results:
The serum calcium level estimated in the blood for different experimental groups are presented in Fig 1 and observation are as follows.

Group-1 (Control):
The changes in plasma calcium content in control group fish for 0, 5, 10, 15,20,25,30 days ranges from 2.15 to 2.20 mg/100ml

Group II (The fish exposed to calcium rich water)
The fish exposed to calcium rich water exhibited increased serum calcium level from 5th day to 15th day and further gradually decreased from 15th day to 30th day indicated that increased absorption of calcium in to blood probably through gills, while gradual decrease in serum calcium after 15 days exposure may be because of corpuscles of Stannius becoming active [for the secretion of hormone stenniocalinin] in regulating blood calcium.

Group III (Calcium rich water + CS extract injected):
The fishes exposed to calcium rich water and CS extract injected have shown increased serum calcium level up to 15th day and later when CS extract injected has reduced serum calcium level indicating that after administration of CS extract [containing the hormone stenniocalcin] reducing the serum calcium level suggesting that CS has a role in the regulation of serum calcium in the freshwater fish Notopterus notopterus.

Histological observations:
The changes in the histological structure of the CS under different experimental conditions has been studied. The CS cells are arranged regularly with prominent nucleus in a control fish (Fig 2). The fish exposed to calcium rich water has exhibited active CS cells showing vesicular type of nucleus and vacuolated cytoplasm (Fig 3). The cells of the CS of the fish exposed to calcium rich water and CS extract injected have also shown hyper active condition (Fig 4) indicating activation of the grandular cells for hormone secretion.

Corpuscles of Stannius responses to distilled water exposure and calcium chloride exposed and injected:
The corpuscles of Stannius (CS) cells under distilled water exposure is 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&2). In calcium chloride exposed and injected fish, the CS exhibits active condition characterised by vesicular nucleus in the majority of cells with cytoplasmic degranulation (Fig. 1&2).

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 (fig.1&2).

Discussion:
The functional role of corpuscles of Stannius in fish remained unsettled for long time. However their role in osmo-regulatory action has been accepted now by the endocrinogists after experimental results from time to time. Now it is acknowledged that Stannius corpuscles produce hormone(s) which restained the development of post stanniectomy hypercalciumia and correct the raised serum calcium which develop after stenniectomy (Fontaine, 1964; Chester Janes et al., 1965;Fenwick,1974.)According to Hirano (1981) it is difficult to observe potential hypocalcimic action of CS in any sate of hyper calcimia other than that caused by stenniectomy especially in eels.

In the present study after exposing the freshwater fish Notopterus notopterus to calcium rich water has shown increase serum calcium, when these fishes injected with CS extract reduced serum calcium indicating that CS extract containing the active compound causes reduction in the serum calcium as reported in other fishes. (Pang et al., 1981).And in Indian fish O.bimaculatus(Pandey,1994) The role of CS in the regulation of calcium homeostasis in fish through the secreation of stenniocalcin a glycoprotein hormone,Witch inhibits transport of gill calcium and also reduces calcium absorption. (Wagner. et al 1989,1991). Secretion of CS depends on calcium as a stimulating factor, This study is based on the intraperitoneal injection of calcium chloride which resulting in CS cell degranulation, in the fish Notopterus notopterus. After exposing fish to calcium rich water upto 15 days the serum calcium level increased and was higher, whereas after 15th day up to 30th day the serum calcium was lower, suggesting that in the freshwater fish Notopterus notopterus also calcium is a regulating factor, is being secreted from CS causing inhibition in the absorption of calcium leading to reduction in the serum calcium level. In the CS extract injected fish the serum calcium level reduces after 15th day bringing to normal level as that of control fish and similar observation has been made in other fishes.(Jun at al.,1989)

The microscopic structures of CS cells have been studied in other type of fishes. After exposing calcium rich water, their results clearly state that calcium environment leads to STC secretion from CS through their observation on cellular details especially enlarged rough endoplasmic reticulum and enhanced exocytosis.(Wandelaar et al., 1976,Pratap&Wandelaar Bonga,2007) In the present study also the CS cells of Notopterus notopterus are active after exposing to calcium rich water and CS extract injected indicating similar kind of hormone (STC) may be secreted from CS cells regulating serum calcium level.As reported in other teleost fishes.

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 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 indicative of inactivation because of absence of calcium in the medium. It is known as that Stenniocalcin hormone of the corpuscles of Stannius involved in the absorption of calcium through gills and intestine of the fish (Lafeber and Perry, 1988; Lefebar 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 and Tikada, 1967; Woodhead, 1968). Hypocalcemia is accelerated in deionised water in the eel (Oliverean 1964). In the present study, when the freshwater fish, N. notopterus exposed to calcium rich water and also injected with calcium solution, the corpuscles of Stannius is active indicating the release of the hormone for calcium homeostasis. This is further supported by 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 release of the hormone, suggesting that calcium is a factor for hormone release from the CS of 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 demonstrated in vitro studies by (Aida et al., 1980) and (Wagner et al., 1989) that increased medium calcium concentrations enhanced the depletion of CS secretary granules and the release of stenocalcin 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 $CaCl_2$ 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.

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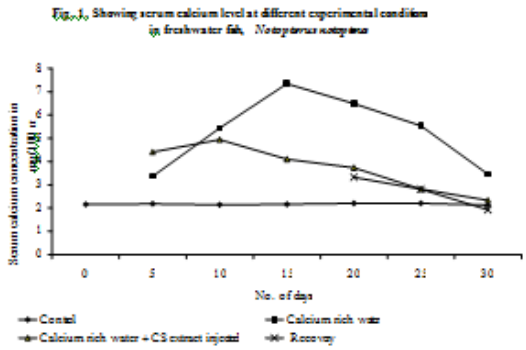

Fig. 1, Showing serum calcium level at different experimental conditions in freshwater fish, *Notopterus notopterus*

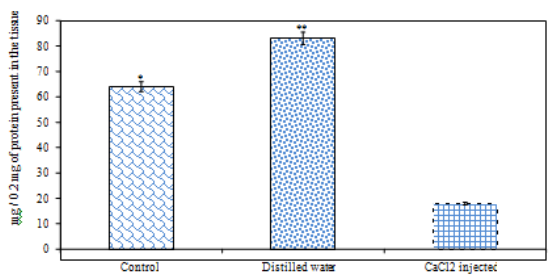

Fig. 2, Showing Protein content of corpuscles of Stannius of Freshwater Fish, *Notopterus notopterus*

REFERENCES

1. A.C.Ded(2002):New Central book agency., Comperehesible viva & Practical Biochemistry., P- 63. | 2. Aida, K., Nishioka, R,S. and Bern, H.A. (1980). Degranulation of the Stannius corpuscles of Coho salmon (Oncorhynchus kisutch) in response to ionic changes in vitro. Gen. comp. Enclocr. 41: 305-313. | 3. Alan, H. Gowenlock, Janet, R. McMurray and Donald, M. McLanchlan (2002) Varley's Practical Clinical Biochemistry, 6th Ed., pg 601. | 4. Belsere, D. K. (1973). Comparative anatomy and histology of Carpuscles Stannius in Teleost. Z.microsk.ann.Forsch. 87, 445-456. | 5. Chester-Jones, I., Henderson,I.W.and Butler, D.G. (1965). Water and electrolyte flux in the European eel (Anguilla Anguilla L.).Arch.Anat.Microsc. Morphol. Exp. 54, 453-469. | 6. Clark, E. P. and Collip, J. B. (1925). Jrnl. Biol. Chem. 63, 461-464. | 7. Cohen, R.S., Pang, P.K.T. and Clark, N.B. (1975). Ultrastructure of the Stannius corpuscles of the killifish, Fundulus heteroclitus, and its relation to calcium regulation. Gen. Comp. Endocr., 21, 413-424 | 8. Fenwick, J.C. and So, Y.P. (1974). A perfusion study of the effect of stanniectomy on the net influx of calcium 45 across an isolated eel gill. Exp. Zool., 188, 125-131. | 9. Fleming, W.R., Stanley, J.G. and Meyer, A.H. (1964). Seasonal effects of external calcium, estradiol and ACTH on the serum calcium and sodium levels of Fundulus kansae. General and Comparative Endo¬crinology, 4: 61-67. | 10. Flik, G., Labedz, T., Lafeber, F.P.J.G., Wendelaar Bonga, S.E. and Pang, P.K.T. (1989a). Studies on teleost corpuscles of Stannius: physiological and biochemical aspects of synthesis and release of hypocalcin in trout, goldfish and eel. Fish Physiol. Biochem., 7: 343-349. | 11. Fontaine, M. (1964) [Stannius' corpuscles and ionic (CA, K, NA) of the interior environment of the eel (Anguilla Anguilla L.)]. C R. Hebd. Seances Acad. Sd. 259, 875-878. | 12. Hanssen, Mayer-Goston, Fiik, G. and Wendelaar Bonga, S.E. (1992). Influence of ambient calcium levels on stanniocalcin secretion in the European eel (Anguilla anguilla), J. Exp. Biol., 162: 197-208. | 13. Hanssen, R.G.J.M., Aarden, E.M., Vander Venne, W.P.H.G., Pang, P.K.T. and Wendelaar Bonga, S.E. (1991). Regulation of the secretion of the teleost fish hormone stanniocalcin; effects of extracellular calcium. Gen. comp. Endocr. (in press). | 14. Hirano,T.,Hasegawe,S.,Yamauchi,H.,&Orimo,H(1981). Further studies on the absence of hypocalcaemic effect of eel calcitonic in the eel, A. japonica. Gen .Comp. Endocrino. 43,42-50. | 15. Jun Ohtsu,and Juro Yamada(1989) Effects of Stannius corpuscle Extract on Calcium influx assayed by Perfusion of Isolated Gills of Rainbow Trout Salmo gairdneri.Bull.Fac.Fish.Hokkaido Univ.40(4),238-245. | 16. Kroshnamurthy, V.G. (1976). Cytophysiology of the CS. Int. Rev.Cytol. 46,177-246. | 17. Lafeber, F.P.J.G. and Perry, S. F. (1988). Experimental hypercalcemia induces hypocalcin release and inhibits branchial Ca2+ influx in freshwater trout. Gen. Comp. Endocr. 72, 136-143. | 18. Lafeber, F.P.J.G., Flik, G., Wendelaar Bonga, S.E. and Perry, S.F. (1988a). Hypocalcin from Stannius corpuscles inhibits gill calcium uptake in trout. Am. J. Physiol., 254, R891-R896. | 19. Milet, C., Peignoux-Deville, J. and Martelly, E. (1979). Gill calcium fluxes in the eel, Anguilla anguilla (L.). Effects of Stannius corpuscles and ultimobranchial body. Comp. Biochem. Physiol. A63, 63-70. | 20. Oguri, M. and Takada, N. (1967). Serum calcium and magnesium levels of goldfish, with special reference to the gonadal maturation. Bull Jpn Soc Scient Fish., 33: 161-166 | 21. Olivereao, M. (1964). Corpuscles of Stannius and osmoregulation in eels. Amer. Zool., 4: 326-327. | 22. Pandey A.C.(1994)Evidence for general hypocalcaemic hormone form Stannius corpuscles of the freshwater catfish.O.bimaculatus.Gen.Comp. Endocrinol. 94(2) 182-185. | 23. Pang, P.K.T., Pang, R.K., Liu, V.K.Y. and Sokabe, H. (1981). Effect of fish angiotensins and angiotensin-like substances on killifish calcium regulation. Ibid. 43, 292-298. | 24. Pratap H.B and Wandelaar Bonga S.E(2007)Calcium Homoeostasis in low and high calcium water acclimated O.mossambicus exposed to ambient and dietary calcium .J.Environ.Biol .28(2)385-93. | 25. So, Y. P. AND Fenwick, J. C. (1979). In vivo and in vitro effects of Stannius corpuscle extract on the branchial uptake of 45Ca in stanniectomized North American eels (Anguilla rostrata). Gen. Comp. Endocr. 37, 143-149. | 26. Wagner, G.F., Gellersen B. and Friesen H.G. (1989) Primary culture of teleocalcin cells from rainbow trout corpuscles of Stannius: Regulation of teleocalcin secretion by calcium. Mol. Cell. Endocrinol., 62,129-138. | 27. Wagner, G.F., Gellersen, B. and Friesen, H.G. (1989). Primary culture of teleocalcin cells from rainbow trout corpuscles of Stannius: Regulation of teleocalcin secretion by Calcium, Molec. Cell. Endocr., 62: 31-39. | 28. Wagner, G.F., Milliken, C., Friesen, H.G. and Copp, D.H. (1991). Studies on the regulation and characterization of plasma stanniocalcin in rainbow trout. Mol. Cell. Endocrinol., 79,129 138. | 29. Wendelaar Bonga, S. E., Greven, J.A.A. and Veenhuis, M. (1976). The relationship between the ionic composition of the environment and the secretory activity of the endocrine cell types of Stannius corpuscles in the teleost Gasterosteus aculeatus. Cell Tissue Res., 175, 297-312. | 30. Wendelaar Bonga, S.E. and Pang, P.K.T. (1991). Control of calcium regulating hormones in the vertebrates: parathyroid hormone, calcitonin, prolactin and stanniocalcin. Int. Rev. Cytol. (in press). | 31. Wendelaar Bonga, S.E. and Perry, S.F. (1988a). Hypocalcin from Stannius corpuscles inhibits gill calcium uptake in trout. Am. J. Physiol. 254, R891–R896. | 32. Wendelaar Bonga, S.E., Van Der Meij, J.C.A. and Pang, P.K.T. (1980). Evidence for secretory cell types in the Stannius bodies of the teleosts Fundulus heteroclitus and Carassius auratus. Cell. Tissue Res. 212, 295-306. | 33. Woodhead, P.M.J. (1968). Seasonal changes in the calcium content of the blood of arctic cod. Journal of Marine Biological Association of the United Kingdom, 48: 81-91. |