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EFFECT OF SYNTHETIC SALMON CALCITONIN ON TISSUE CALCIUM LEVELS IN THE BAGRID CATFISH, MYSTUS GULIO (HAMILTON)

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SUMMARY

Effect of synthetic salmon calcitonin (SCT) on tissue calcium (Ca) level was studied in euryhaline catfish *Mystus gulio*. Intraperitoneal administration of 20, 40 and 60 i.u. of SCT/fish/day for 5 days produced transient but significant hypocalcemic responses in blood, ovary and air bladder. The hypocalcemia was sustained for about 24 hours post injection and the animals regained normocalcemia in about 48 hours. During the same period, transient but significant hypercalcemic responses were observed in the bone and muscle of SCT treated fish that regained normocalcemia. The hypocalcemic or hypercalcemic response in the respective tissues was found to be dose dependent. The air-bladder was found to be a significant store house/supply centre for Ca and showed marked bleaching due to Ca discharge/erosion on prolonged calcitonin treatment. The same was reflected in the Ca content. The above results obtained in *Mystus gulio* suggest that the Ca homeostasis is efficient in this species and that calcitonin acts not only on bones but also on tissues like muscles, ovary and air-bladder.

Key words: Air-bladder; Bone; Hypercalcemia; Hypocalcemia; Muscle; *Mystus gulio*; Ovary; Synthetic Salmon Calcitonin.

INTRODUCTION

Calcium (Ca) balance in vertebrates is regulated by an interaction among hormonal factors like parathyroid hormone (PTH), stanniocalcin, calcitonin (CT), vitamin-D, prolactin, somatotropin, corticosteroids, estrogens, thyroxine, and glucagon (1). Of the aforementioned hormones, CT displays variant properties especially in fishes in its Ca regulating tendency. While several workers (2-9) have attributed a blood hypocalcemic action to CT in fish, they have been contradicted by others (10-12) who endorse a hypercalcemic action to CT. A third front to this argument is presented by some (10, 13-16) in whose studies CT administration failed to produce any significant effect on Ca levels. The present study aims to determine the effect of CT on the tissue Ca levels in the euryhaline Bagrid teleost, *Mystus gulio* in an attempt to elucidate the role of this hormone in Ca regulation.

MATERIALS AND METHODS

Adult females of *Mystus gulio* were collected from the backwaters of Ogyampet, situated about 25 km south of the city on Chennai between August to October. They were maintained in aerated plastic tanks (91 x 6 x 61 cm) after suitable acclimatization to bore-well water.

Fifty-four fish of similar size and weight were divided into six lots of nine each, three serving as experimental groups and the other three serving as controls. Fish of the first, second and third lots were each injected intraperitoneally with 20, 40 and 60 i.u. respectivly, of synthetic salmon calcitonin (SCT; Zycalcit 100; 100 i.u. potency / ml) daily for five days with intervals of 24 h. The respective control groups were injected with equal volumes of physiological saline at the same time. Three fish from each group were sacrificed 24 h, 48 h and 72 h after the last injection and the Ca level was estimated in the blood, bone, muscle, ovary and air-bladder.

Blood samples was obtained from an incision at the caudal peduncle and collected in a heparinized test tube. Samples of vertebral bone, muscle from the peritoneal wall, air-bladder and ovary were collected and weighed. Exception for the blood, all tissues were homogenized and centrifuged at 10,000 rpm for 15 minutes and the supernatant collected for estimating Ca content.

The Ca level in blood and tissues was estimated by O-cresolphathalein complexone method according to Bauer (17). Analysis of variance (ANOVA) was performed to determine the statistical significance of the differences between means at 0.05 level. The significant difference between any two means was obtained by studentized Q-test (18). When the differences among the control means were not significant, combined mean and standard deviation were calculated.

RESULTS AND DISCUSSION

The results obtained in the euryhaline catfish *Mystus gulio* following injection of multiple doses of SCT clearly indicate that this hormone has hypocalcemic effect in blood, ovary and air-bladder, and hypercalcemic effect in bone and muscle (Table 1). Further, it is seen that the change in the calcium levels is only transitory, suggesting that this species of fish possess an efficient mechanism of Ca homeostasis.

Hypocalcemic action of CT has been reported in *Anguilla anguilla* (2, 3, 7), *Channa punctatus* (4), *Carassius auratus* and *A. anguilla, inter alia* (6). Results of the present study also suggest a hypocalcemic effect of CT in the teleost, *Mystus gulio.* Although few studies (6, 10) have shown the effect of CT on calcium level in various fish species it is difficult to draw any conclusion as there is no clear indication about the actual intraperitoneal load of CT in experimental animals in the corresponding time allowed at the time of experimentation. Hence, general trends alone are discussed as they are in good agreement.

Table 1 : Effects of daily injections of synthetic salmon calcitonin for five days on the calcium levels in blood, bone, muscle, ovary and air bladder of *Mystus gulio*

Dosage ml/fish/ Day	Hours After Injection	Blood (mg/dl)		Bone (mg/g)		Muscle (mg/g)		Ovary (mg/g)		Air-bladder (mg/g)	
		Control	Expt.	Control	Expt.	Control	Expt.	Control	Expt.	Control	Expt.
0.2	24	18.80 ± 0.16	18.20 * ± 0.08	4.50 ± 0.08	5.20 * ± 0.08	3.10 ± 0.08	3.50 ± 0.08	6.26 ± 0.04	5.52 * ± 0.08	4.10 ± 0.08	3.73 ± 0.04
	48	18.73 ± 0.12	18.13 * ± 0.16	4.66 ± 0.12	4.96 ± 0.47	3.26 ± 0.12	3.80 * ± 0.08	6.30 ± 0.14	5.33 * ± 0.12	4.13 ± 0.09	3.63 * ± 0.12
	72	18.72 ± 0.08	18.70 ± 0.14	4.66 ± 0.16	4.63 ± 0.12	3.20 ± 0.02	3.26 ± 0.12	6.16 ± 0.09	6.20 ± 0.04	4.16 ± 0.09	4.06 ± 0.47
0.4	24	18.00 ± 0.08	17.21 * ± 0.12	4.63 ± 0.16	5.57 * ± 0.08	3.20 ± 0.08	3.73 * ± 0.47	6.40 ± 0.08	5.40 * ± 0.04	4.36 ± 0.12	3.40 * ± 0.08
	48	17.90 ± 0.08	17.30 * ± 0.08	4.86 ± 0.12	5.30 ± 0.21	3.16 ± 0.16	3.63 ± 0.12	6.36 ± 0.16	5.20 * ± 0.08	4.30 ± 0.08	3.43 * ± 0.16
	72	17.72 ± 0.08	17.70 ± 0.16	4.63 ± 0.30	4.90 ± 0.16	3.20 ± 0.20	3.20 ± 0.08	6.26 ± 0.16	6.20 ± 0.08	4.33 ± 0.16	4.06 ± 0.04
0.6	24	18.06 ± 0.12	17.12 * ± 0.08	4.56 ± 0.16	5.71 * ± 0.08	3.30 ± 0.08	3.93 * ± 0.47	6.43 ± 0.04	5.14 * ± 0.08	4.36 ± 0.47	3.06 ± 0.08
	48	18.16 ± 0.12	17.30 * ± 0.16	4.70 ± 0.08	5.80 * ± 0.08	3.30 ± 0.12	3.96 * ± 0.12	6.33 ± 0.12	5.00 * ± 0.16	4.46 ± 0.04	3.20 * ± 0.08
	72	17.80 ± 0.04	17.83 ± 0.04	4.54 ± 0.16	4.50 ± 0.12	3.30 ± 0.21	3.36 ± 0.08	6.26 ± 0.12	6.20 ± 0.12	4.46 ± 0.12	4.10 ± 0.08

Each value is mean ± S.D. n = 3. * $p < 0.05$

The intestine, kidney, gills and scales are important organs of Ca metabolism (10). Bonga and Lammers (19) failed to notice any change in the Ca concentration of bone with CT treatment in Sarotherodon mosambicus. However, in the present study, appreciable changes in bone calcium level was observed in *Mystus gulio.* Lopez *et al.* (20) have reported that CT has no effect on blood Ca levels but it prevents bone demineralization in *Salmo gairdneri.* This may be due to the fact that CT totally prevents or reduces Ca resorption from storage reservoirs as opposed to the stimulation or promotion of PTH (21). Blood Ca levels are good indicators of Ca gain or loss of the body Ca pool. The fall in blood Ca level in *Mystus gulio* observed in the present study, could be attributed to the hypocalcemic action of CT. The Ca removed from the blood would have to be deposited on some other tissue or excreted through the gills and kidneys. CT has been shown to enhance renal Ca excretion, stimulate branchial Ca efflux and to reduce branchial Ca influx (2). In the present study, the low calcium level in blood may be either due to increased uptake of Ca by bone and muscle tissues or due to excretion through gills or kidney (Table-1).

Interestingly, the air bladders of the CT treated fish were found to be bright white compared to the dull, yellowish color observed in normal fish. On analysis, the Ca level in the air-bladder of treated fish was found to be lower than that in normal ones. This suggests that the air bladder too acts as a Ca depositing center or reservoir at times of high Ca availability or influx (22). The action of CT on the air bladder invites further and deeper investigation.

Björnsson *et al.* (23) and Norberg *et al.* (24) suggested that CT helps in ovarian maturation and does not reduce levels of circulating Ca necessary for vitellogenesis and related processes. In the present study, high doses of CT (40 i.u. and 60 i.u./fish) were found to deplete ovarian Ca by exercising its hypocalcemic property. This depletion is not in accord with the earlier studies (2-4, 7) as Ca depletion from the ovaries would work against vitellogenesis. The discrepancy between results of the present study and early reports may be due to variations in experimental designs. It needs further study to arrive at a definite conclusion. However, the present study has opened up the idea that the ovaries are also the target organs of CT.

As Ca level increases in muscle tissue on CT administration as in bone tissue, it is inferred that CT protects muscle tissue from loosing Ca. As a general rule, trends of bone and muscle tissue Ca levels coincide suggesting that structural tissues do not loose Ca with CT treatment. In *Mystus gulio,* it is observed that the effects of CT are dose dependent.

An important factor which is bound to affect the level of calcium and therefore, the CT level in the blood is the level of Ca in the ambient medium. High external environmental Ca concentration was found to reduce the plasma calcitonin level in the immature brown trout, *Salmo trutta* (12). A decline in plasma calcitonin has also been observed in the rainbow trout, *S.gairderi*, transferred from freshwater to seawater, though this response was preceded by an increase in the hormone concentration (11). Such studies in Japanese eel, *Anguilla japonica* (16) and coho salmon, *Oncorhynchus kisutch* (25), however, failed to show any changes in plasma

CT concentration. In the present study, the backwater medium from which the catfish were collected and the bore-well water in which they were maintained in the laboratory had almost similar concentrations of calcium (22). Therefore, any change observed in the blood Ca concentration in *Mystus gulio* due to variations in the environmental Ca levels may be ruled out. Unfortunately, we could not measure CT level. Nevertheless, our results indicate that the effect of CT in *Mystus gulio* is organ specific.

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