THE EFFECTS OF SYNTHETIC SALMON CALCITONIN ON THE HISTOMORPHOLOGY OF THE INTERRENAL IN THE BAGRID CATFISH MYSTUS GULIO (HAM)

B. Radha, B. Deivasigamani and V. Ramanathan
Department of Zoology, Pachaiyappa’s College, Chennai - 600 030, India

ABSTRACT
The histomorphology of the Interrenal (IR) of Mystus gulio showed hypertrophic changes 24 hours after the last injection of varying dosages of Synthetic Salmon Calcitonin (SCT). The hypertrophic symptoms were increase in the size of the cells and nuclei and lighter staining of the cytoplasm due to depletion of secretory granules. The nucleoli appeared conspicuous, suggesting increased synthetic activity. These changes in the IR might be a direct effect of the alterations in the calcium concentration in blood or a result of the dearth of ovarian hormones consequent to ovarian atrophy.

INTRODUCTION
The entire web of hormonal calcium (Ca) homeostasis in fishes, especially females, comprises of calcitonin (CT) of ultimobranchials, stanniocalcin of corpuscles of Stannius (CS), prolactin (PRL) of hypophysis and the hormonal products of the interrenals (IR) and ovary (Bjornsson et al., 1986, 1989; Fouchereau-Peron et al., 1990; Bentley, 1998). A perusal of the pertinent literature shows that the CS, IR and ovary are bound to be affected by any natural or induced changes in Ca and other calcemic hormone levels in the body (Heyl, 1970; Janakiraman, 1998; Aida, et al., 1980; Hanssen et al., 1991; Urasa and Wendelar, 1987). Earlier it was observed in Mystus gulio (Ramanathan, 1998) that CT administration brought about significant calcemic changes in the Ca levels of blood, bone, muscle, ovary and air-bladder. The present study reports the histomorphological changes observed in IR of Mystus gulio in response to exogenous administration of CT.

MATERIAL AND METHODS
Adult females of Mystus gulio, collected from the backwaters of Ogampet, about 25 km south of Chennai, during August to October, were maintained in aerated plastic tanks after suitable acclimatization to bore-well water. A group of fifty-four mature females of similar size and weight was used for the study. The fish 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 0.2 ml, 0.4 ml and 0.6 ml respectively of synthetic salmon calcitonin (SCT; Zycalcit 100 of 100 i.u./ml) daily for five days with intervals of 24 h while the respective control groups were injected with equal volumes of physiological saline at the same time.

The IR of the experimental and control fish were fixed in Bouin’s fluid, processed, paraffin-embedded, cut into 6 mm thick sections and stained in hematoxylin and eosin (HE). Experimental and respective control groups were compared for changes in the cytoplasmic characters and cyto-karyometric measurements. The diameters of 25 randomly chosen cells and their nuclei were measured using ocular micrometer. Analysis of variance (ANOVA) was performed for the combined experimental and control groups in order 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 (Snedecor and Cochran, 1967).
RESULTS AND DISCUSSION

The histomorphology of the IR of Mystus gulio has been described in detail earlier (Janakiraman, 1998, Ramanathan, 1998). The following report is, therefore, confined to changes observed in the organ in the present experiment.

**Interrenals:** The IR cytoplasm is distinctly eosinophilic and intensely stained due to the presence of fine secretary granules. The degree of staining varies considerably depending on the concentration of secretory granules. Prominent nuclei with conspicuous single nucleoli characterized the IR cells. The nuclei were generally round. The chromatin material definition ranged from diffuse to well defined states depending on the functional status of the cell and nucleus. The histomorphological structure of the IR showed definitive changes after treatment with SCT in varying doses (0.2, 0.4 and 0.6 ml per fish) for 5 days. These changes pointed to a graded progress towards individual cell and whole organ hypertrophy.

The control tissue showed darkly staining dense nuclei, centralized and taking up the major part of cell volume, the cells being small and compactly arranged (Figs. 1 and 2) and the nucleoli indistinct. The experimental IR tissue exhibited signs of hypertrophy increasingly expressed with increasing doses. The cells became larger, turgid and stained lighter than those in controls (Figs. 3 and 4). The nuclei appeared to be increased in size. Nucleoli were conspicuous and the nucleus occupied larger part of cell volume than in the control as the cells themselves appeared much inflated in size. However, results of the cyto-karyometric features (Table 1) of the IR cells were inconsistent, as also was in the case of CS. Statistical significance of the difference between experimental and control values could be found only in a few cases.

The histomorphological changes in IR of SCT treated Mystus gulio discussed hereunder have relevance to the calcemic changes observed (Ramanathan, 1998) in blood, bone, muscle, ovary and air-bladder of this species of euryhaline catfish under the same experimental conditions. SCT administration in multiple doses was found to cause transient but significant hypocalcemia in blood, ovary and air-bladder and hypercalcemia in bone and muscle.

**Table 1.** Effects of multiple injections of calcitonin in varying doses on the cyto-karyometric features of the IR of Mystus gulio

<table>
<thead>
<tr>
<th>Dosage (ml/fish/day last injection)</th>
<th>Hours after last injection</th>
<th>IR Cell diameter X ± S.D. (µm)</th>
<th>IR Nuclear diameter X ± S.D. (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Expt</td>
<td>Control</td>
</tr>
<tr>
<td>0.2</td>
<td>24</td>
<td>5.31±0.07</td>
<td>4.89±0.05</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>6.04±0.05</td>
<td>5.69±0.05</td>
</tr>
<tr>
<td>0.4</td>
<td>24</td>
<td>6.50±0.16</td>
<td>5.69±0.05</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>6.24±0.13</td>
<td>6.14±0.09</td>
</tr>
<tr>
<td>0.6</td>
<td>24</td>
<td>6.76±0.13</td>
<td>5.77±0.09</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>8.82±0.19</td>
<td>5.38±0.11</td>
</tr>
</tbody>
</table>

ANOVA 'F' 568.33 39.18
Q-test : Significant difference 0.43 0.84

* Differ significantly from the respective control; P < 0.05.
Fig. 1. Showing the distribution of IR (arrow) around the posterior cardinal vein (PCV) in a control fish. IR is surrounded by lymphocytes (L). X 150

Fig. 2. Showing polygonal IR cells with eosinophilic cytoplasm containing fine secretory granules in a control fish. C, capillary. X 750
Fig. 3. Showing IR (arrow) of SCT treated (0.6 ml/fish/day for 5 days) fish with lightly staining cells. C, capillary; L, lymphocytes; PTV, posterior cardinal vein; X 160

Fig. 4. Showing higher magnification of IR of SCT treated fish. The cells and nuclei appear enlarged, nucleus conspicuous, cytoplasm degranulated. C, capillary. X 750
The IR is also implicated in osmoregulation and mineral regulation in *Anguilla anguilla* (Olivereau and Olivereau, 1970), and in *Cyprinus carpio* and *Sarotherodon mossambicus* (Hegale and Hanke, 1984).

In the present study involving the euryhaline teleost *Mystus gulio* adapted to freshwater, signs of hypertrophy of the IR are evident following SCT administration. Though the IR follows the ovary in functional status according to the trend of the reproductive condition, it does not atrophy as the ovary does in response to exogenous SCT administration. This can be explained as a direct effect of the change in Ca concentrations as doctored by CT or the dearth of the ovarian hormones consequent to ovarian atrophy. The freshwater medium being of lower Ca concentration than the internal milieu of the fish would naturally make the IR hyperactive so that the fish would not lose much Ca. To add to this, the drain of Ca by CT would put additional stress on the IR. Hence hypertrophy of the IR, whereby greater secretion is achieved to help in the inward transport of Ca for homeostasis is the only logical recourse.

**REFERENCES**


