Effects of calcium and phosphate on the corpuscles of Stannius of the teleost fish, Oreochromis mossambicus

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Summary. Removal of the corpuscles of Stannius (CS) in Oreochromis mossambicus leads to hypercalcemia and hypophosphatemia. The effects on CS size and ultrastructure of different calcium and phosphate concentrations of the ambient water and of the food were investigated. A six-fold increase of the calcium concentration of the water leads to a four-fold increase in CS volume; this is mainly caused by an increase in the size and number of the type-1 cells. The effect of external calcium is most probably mediated by the calcium concentration of the blood plasma. Plasma ionic calcium may be the relevant factor. Changes in the calcium concentration of the food had no effect on the CS. Similarly, hyperphosphatemia or hypophosphatemia induced by high phosphate concentrations of the water or the food, or by a phosphate-deficient diet, had no noticeable effect on the CS. The results support the hypothesis that the type-1 cells produce the hypocalcemic factor of the CS. There is no evidence for the production by the CS of an endocrine factor involved in the control of phosphate metabolism.

Key words: Stannius corpuscles – Endocrine control of calcium metabolism – Teleost fish – Blood calcium and phosphate – Oreochromis mossambicus

The corpuscles of Stannius (CS), small endocrine glands in the kidneys of holostean and teleostean fish, have recently become the subject of renewed interest because they may be homologous with the parathyroid glands of terrestrial vertebrates. Substances produced in the CS have immunological similarities with parathyroid hormone (PTH; Milet et al. 1980; Lopez et al. 1984). Injection of CS extracts in rats stimulated osteoclastic resorption and induced calcium release in rat fetal bones in vitro (Milet et al. 1979). We have also shown that CS extracts exert appreciable PTH-like activity when applied to mouse bone in vitro, such as induction of calcium and phosphate release, the stimulation of lactate production, and the activation of osteoclasts (Lafeber et al. 1986). Conversely, PTH has been shown to induce hypocalcemia in fish (Lafeber et al. 1984; Wendelaar Bonga et al. 1986). This effect is also achieved using the active principle(s) of the CS (Pang et al. 1973, 1974; Wendelaar Bonga et al. 1986).

The action of PTH in terrestrial vertebrates is not limited to its well-known hypercalcemic activity. This hormone also affects phosphate metabolism, in particular by inducing hyperphosphatemia and stimulating renal phosphate excretion (Parsons 1979). Fontaine (1964, 1967) was the first to demonstrate that removal of the CS (stanniectomy) in eels leads to a prolonged and marked increase in plasma calcium levels, and reported that plasma phosphate levels were also affected. The decrease he observed has since been confirmed (Chan and Chester-Jones 1968; Chan 1969, 1972), but the possibility that the CS are involved in the endocrine control of phosphate metabolism has received little attention. The hypophosphatemia observed after removal of the CS indicates direct or indirect involvement of the CS in phosphate metabolism. The CS may produce a hyperphosphatemic factor that may be identical to the hypocalcemic factor or a separate entity. It is of interest that the CS of many teleost species contain two structurally distinct cell types (Krishnamurthy and Bern 1968; Wendelaar Bonga and Greven 1978; Wendelaar Bonga et al. 1980; Meats et al. 1978). Thus, more than one hormonal substance may be produced by the CS. Heterogeneity of the CS secretory products is also suggested by the results of biochemical analysis of the CS. During the incubation of trout CS in vitro, a molecule with an apparent molecular weight of 25000 to 28000 dalton, and a smaller product of about 13000 dalton are synthesized and released by the CS (Lafeber et al. 1984, 1986; Wendelaar Bonga et al. 1985). Recently, Wagner et al. (1986) have isolated a glycopeptide, with an estimated molecular weight of 39300 dalton, from the CS of trout. Lopez et al. (1986) have isolated a 34000-dalton protein with hypocalcemic properties from eel CS, and a 70000-dalton protein with characteristics of the SP-1 cosecretory protein present in mammalian parathyroid glands.

In the present study, the possible involvement of CS in phosphate metabolism is explored by comparing the effects of different calcium and phosphate loads on the size and ultrastructure of the CS in an African cichlid teleost fish, Oreochromis mossambicus. Firstly, we show that removal of the CS in O. mossambicus changes both plasma calcium and phosphate levels. Secondly, we have analyzed the effects of different calcium and phosphate concentrations of the ambient water on plasma calcium and phosphate, on the size of the CS, and on the ultrastructure of the two endocrine cell types of the CS, the type-1 and the
type-2 cells. For *O. mossambicus*, the main source of calcium is water (Flik et al. 1985a), and the main source of phosphate is food (Flik et al. 1985b). We have therefore examined the effects on the CS of different calcium and phosphate concentrations in the food.

### Materials and methods

Freshwater male *Oreochromis mossambicus* (formerly *Tilapia mossambica* and *Sarotherodon mossambicus*) were used; they varied in body weight between 10 and 15 g. They were kept in groups of about 8 fish in 100-l full glass aquaria, at 25°C, in tapwater containing 2.1 mM Na⁺, 0.5 mM Cl⁻, 0.4 mM SO₄²⁻, 0.8 mM Ca²⁺ and less than 0.01 mM inorganic phosphate.

**Removal of the CS.** The fish were anaesthetized in MS 222. The peritoneal cavity was opened by an incision in the lateral body wall. The caudal area of the kidneys was dissected free and gently tilted, to expose the CS. In shamoperated fish, the body wall was closed at this stage of the operation. For stanniectomy the glands were removed carefully using fine forceps. The incisions in the body walls were sutured. The fish were replaced for three days in fresh water containing 3.5 g/l NaCl, and afterwards in normal fresh water. Eight days after the operation the fish were anesthetized in MS 222, and the blood was collected for the determination of total and free calcium and phosphate levels.

**Exposure to different calcium and phosphate concentrations.**

Artificial tap water was prepared according to Wendelaar Bonga and Van der Meij (1980). Calcium was added as CaCl₂ to obtain Ca²⁺ concentrations of 0.1, 0.2, 0.4, 0.8, 2.5, and 5.0 mM. The fish were exposed to the following concentrations of Ca²⁺: 0.4 mM for 2 days, 0.2 mM for 2 days, and finally 0.1 mM for 5 weeks; or 0.4 mM for 2 days and then 0.2 mM for 5 weeks. Some fish were transferred directly to water of the final concentration. The water was replaced weekly.

High-phosphate water (Ca²⁺: 0.8 mM) was obtained by adding a 1 M solution of NaH₂PO₄ and Na₂HPO₄ (pH: 7.4) to artificial water, to make a final concentration of 2.5 or 5.0 mM H₂PO₄⁻/HPO₄²⁻. The water was replaced every week. The fish were exposed to one or other of these solutions for 5 weeks.

**Food with different calcium or phosphate concentrations.** Artificial food was prepared following Berg’s (1968) recipe, with slight modifications. Casein was replaced by egg albumen (Ichii and Mugiya 1982). The concentration of phosphate was 0.025 mmol·g⁻¹ dry weight in the phosphate-deficient food, 3.50 mmol·g⁻¹ dry weight in the high-phosphate food, and 0.69 mmol·g⁻¹ dry weight in the control food. The concentration of calcium was 0.01 mmol·g⁻¹ dry weight in the calcium-deficient food, 1.51 mmol·g⁻¹ dry weight in the high-calcium food, and 0.27 mmol·g⁻¹ dry weight in the control food. The fish were fed these diets for 6 weeks. The water was replaced every week.

**Blood sampling and dissection.** At the end of the experiments, the fish were anaesthetized lightly in MS 222, and weighed. Blood was collected from the caudal blood vessels after cutting the caudal peduncle. Plasma was obtained by centrifugation. Some plasma was ultrafiltered using a Sar-
Electron microscopy. The CS were dissected out and prefixed for 10 to 15 min in 3% glutaraldehyde buffered with 0.1 M cacodylate buffer, pH 7.4, at room temperature. The CS were then fixed for 1 h at 0°C in a solution containing 2% osmium tetroxide, 1.5% glutaraldehyde and 2.5% potassium dichromate in 0.1 M cacodylate buffer. Postfixation was carried out in 2% uranyl acetate. After dehydration in ethanol the tissues were embedded in Spurr’s resin. Ultrathin sections were poststained with Reynold’s lead citrate and examined in a Philips EM 200 electron microscope.

Morphometric analysis. The CS of 5 fish per group were prepared for electron microscopy. Micrographs at a final magnification of 13000×, representing a sampling area of 500 μm² per cell type per fish, were analyzed morphometrically with Kontron Digiplan equipment. Details of the procedure have been published (Urasa and Wendelaar Bonga 1985).

Statistical analysis. The data on plasma electrolytes were statistically analyzed using Kruskal-Wallis one-way analysis of variance; the data on CSI and the morphometric data were analyzed using the Mann-Whitney U-test (two-tailed). Significance was accepted at the 5% level.

Results

1. Stanniecotomy

Removal of the Stannius bodies led to an increase in the total plasma calcium and in the ultrafiltrable, non-protein-bound plasma calcium fraction (Table 1). A reduction was observed in the total and ultrafiltrable plasma phosphate concentrations, although the reduction of the latter was not significant.

2. Effects of calcium

Water calcium concentration. An increase of the calcium concentration of the water leads to an increase in the size of the CS (Fig. 1). The difference in CSI between fish from 5 mM Ca²⁺ and the controls (0.8 mM Ca²⁺) is statistically significant.

<table>
<thead>
<tr>
<th>Type-1 cells</th>
<th>Type-2 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>High-calcium</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td></td>
<td>High-phosphate</td>
</tr>
<tr>
<td><strong>Cell area (μm²)</strong></td>
<td>23.41 ± 2.76</td>
</tr>
<tr>
<td><strong>Nuclear area (μm²)</strong></td>
<td>8.45 ± 1.84</td>
</tr>
<tr>
<td><strong>Mitochondria (vol. %)</strong></td>
<td>4.02 ± 1.29</td>
</tr>
<tr>
<td><strong>Golgi area (vol. %)</strong></td>
<td>5.24 ± 1.41</td>
</tr>
<tr>
<td><strong>Granular ER (vol. %)</strong></td>
<td>14.83 ± 2.18</td>
</tr>
</tbody>
</table>

* Significantly different from controls, P < 0.05
** Significantly different from controls, P < 0.01
Fig. 3. Type-1 cells (t-1), with large secretory granules, and type-2 cells (t-2), with small secretory granules (arrows) of CS of controls (water calcium concentration: 0.8 mM); ger granular endoplasmic reticulum; Ga Golgi areas; c connective tissue; bar represents 1 μm.

significant ($P < 0.001$). A decrease in the calcium concentration to 0.2 mM Ca$^{2+}$ also leads to a significant increase of the CSI ($P < 0.01$). In contrast, a further decrease of water calcium to 0.1 mM leads to a significant reduction of the CSI, when compared to water with 0.2 mM Ca$^{2+}$ (Fig. 1). The changes in the plasma calcium levels induced by the variation of the external Ca$^{2+}$ levels parallel the changes in the CSI. This is true for the total plasma calcium concentration and the ultrafiltrable plasma calcium fraction (Fig. 2). Plasma phosphate levels are unchanged (Fig. 2).

The general structure of the CS and the ultrastructure of the two endocrine cell types that constitute the CS of O. mossambicus have been described (Urasa and Wendelaar Bonga 1985). To determine the cell type(s) responsible for the increase in size of the CS at 5 mM Ca, the CS cells of the fish concerned were morphometrically analyzed and compared with control fish. The results (Table 2) show changes in the type-1 cells only. This cell type, which makes up about 55% of the volume of CS in control fish (Figs. 3, 6), provides 85% of the volume of CS in the fish exposed to high-calcium water (Figs. 4, 6). The cell size, Golgi area, and volume density of the granular endoplasmic reticulum are significantly larger than in control type-1 cells (Table 2). The volume density of the mitochondria is similar to that in the controls; this implies that the volume of mitochondria per cell increases to the same extent as the cell volume, i.e. by more than 80%. No morphometrical differences are observed between the type-2 cells of both groups of fish (Table 2).

The fractional volume of the type-1 cells shows a variation in relation to the water calcium concentration (Fig. 6) that is similar to that of the plasma total and ultrafiltrable calcium concentration (Fig. 2). At 0.1 mM Ca$^{2+}$, the percentage of type-1 cells is significantly reduced when compared to the control value. Ultrastructural examination shows that the type-1 cells are small and degranulated, with reduced Golgi areas and granular endoplasmic reticulum (Fig. 5).

Calcium-deficient and high-calcium food. Neither a reduction of the calcium concentration of the food by more than 95%, nor a five-fold increase of this concentration noticeably affects plasma calcium- or phosphate levels, the CSI (Table 3), or the ultrastructure of the endocrine cells of the CS (not shown).

3. Effects of phosphate

Water phosphate concentration. Exposure of fish to water containing 2.5 mM or 5 mM phosphate does not noticeably influence the size of the CS (Fig. 1), although mean plasma total phosphate and plasma ultrafiltrable phosphate levels are both slightly elevated (Fig. 7). The difference as compared with controls is statistically significant for the total phosphate level in the 5-mM phosphate group.

Morphometrical analysis at the ultrastructural level does not reveal any differences in type-1- and type-2 cells...
Fig. 4. Type-1 (t-1) and type-2 (t-2) cells of CS of fish adapted for 5 weeks to high-calcium freshwater (water calcium: 5 mM). The type-1 cells are large, with extensive granular endoplasmic reticulum (ger) and a large Golgi area (Ga); bar represents 1 μm.

Fig. 5. Type-1 (t-1) and type-2 (t-2) cells of CS of fish adapted for 5 weeks to low-calcium freshwater (water calcium: 0.1 mM. The type-1 cells are small and their secretory granules are scarce; ger granular endoplasmic reticulum; Ga Golgi area; lys lysosome; c blood capillary; bar represents 1 μm.
Fig. 6. Effect of the calcium (dots) or phosphate (squares) concentration of freshwater (FW) on the fractional volume (%) contributed by type-1 cells to the total volume of endocrine cells of the CS (100% = total volume of type-1 and type-2 cells). The fractional volume was estimated by determining the contribution of type-1 cells to the total surface area occupied by type-1- and type-2 cells in electron micrographs of CS ultrathin sections; // = 6; * significantly different from controls (water calcium concentration: 0.8 mM), $P < 0.05$, ** $P < 0.01$ between phosphate-treated fish and controls (Table 2). The volume ratio of the type-1- and type-2 cells (Fig. 6), and the ultrastructure of these cells (Fig. 8) are unchanged.

Phosphate-deficient and high-phosphate food. Reduction of the phosphate content of the food by more than 95% does not influence the size of the CS (Table 3) or the ultrastructure of their endocrine cells (Fig. 9). Plasma total and ultrafiltrable phosphate levels are reduced by about 10% (Table 3). However, the differences as compared with the controls are not statistically significant. Plasma calcium levels are unchanged. A five-fold increase of the phosphate concentration of the food leads to a significant hyperphosphatemia (Table 3), but CSI (Table 3) and the ultrastructure of the endocrine cells of the CS (not shown) are not noticeably influenced.

Discussion

Stanniaectomy. Removal of the Stannius bodies in O. mossambicus results in hypercalcemia, similar to that observed in other teleost fish following stanniectomy, viz., several species of eels (Fontaine 1964; Chan 1969; Bailey and Fenwick 1975), goldfish (Ogawa 1968), killifish (Pang 1971), sticklebacks (Wendelaar Bonga and Greven 1978) and the Chilean clingfish (Galli-Gallardo et al. 1977). In O. mossambicus the hypercalcemia is associated with a slight hypo-

phosphatemia. A similar reduction of plasma phosphate has been reported for European and Japanese eels (Fontaine 1967; Chan 1972).

CS and calcium. An increase of the $Ca^{2+}$ concentration of the water, from 0.8 to 5.0 mM, leads to significant growth of the CS. Reduction of the $Ca^{2+}$ concentration to 0.2 mM also stimulates CS growth, whereas a further decrease is followed by involution of the CS. This unexpected and complicated relationship between the $Ca^{2+}$ level of the water and the CS indicates that the effects of external $Ca^{2+}$ on the activity of the endocrine cells of the CS are mediated by the plasma calcium concentration: our data show that there is a direct relationship between the plasma calcium level and the size of the CS, over the whole range of water $Ca^{2+}$ concentrations tested. The rise in plasma calcium in O. mossambicus following reduction of the water $Ca^{2+}$ concentration from 0.8 to 0.2 mM has been observed before in our laboratory (Flik et al. 1985a). At the lower concentration the fish consume more food and display a higher growth rate. When the water $Ca^{2+}$ level is reduced further, the fish are apparently unable to maintain plasma calcium levels, and the resulting drop in plasma calcium is associated with reduction in the size of CS.

Increased CS activity during hypercalcemia induced by exposure to high-calcium water has previously been reported in catfish (Suryawanshi and Mahajan 1976) and sticklebacks (Wendelaar Bonga et al. 1976). However, no effect is observed in goldfish after exposure to high-calcium water (Umehara and Oguri 1978). Interestingly, in the latter
Fig. 8. Type-1(r-1) and type-2 (r-2) cells of CS of fish adapted for 5 weeks to water containing 5 mM phosphate; bar represents 1 μm.

Fig. 9. Type-1(r-1) and type-2 (r-2) cells of CS of fish on a phosphate-deficient diet for 6 weeks; bar represents 1 μm.
experiment no increase in plasma calcium concentration is found. This is in line with our conclusion that the effects of water calcium levels on the CS are indirect and mediated by plasma calcium. This conclusion also agrees with in vivo observations of Lopez et al. (1985), who have shown that calcium chloride injections in eels lead to degranulation of the CS cells. Aida et al. (1980) have found that salmon CS, when incubated in vitro, are degranulated in response to an increase of the ionic calcium concentration of the incubation medium. The latter observation indicates that it is the ionic calcium fraction of the plasma that determines CS activity. Our present data are inconclusive in this respect, because the plasma total calcium and plasma ultrafiltrable calcium fraction show the same change in response to variation of the water Ca\(^{2+}\) concentration as the CSI. However, in an earlier study (Urasa and Wendelaar Bonga 1985), we have shown that the six-fold increase in plasma protein-bound calcium during ovarian maturation of female *O. mossambicus* is accompanied by an increase of the CSI of less than 35%. The present study shows that in fish exposed to water containing 5 mM Ca\(^{2+}\) there is an increase of only 25% in protein-bound calcium, whereas the CSI increases by more than 75%. Thus, there seems to be no relationship between plasma protein-bound calcium and the CSI. The results of Aida et al. (1980), indicating that ionic calcium is an important factor in the control of hormone release from the CS, and the finding that CS extracts reduce in particular the plasma ionic calcium fraction (Bailey and Fenwick 1975), raise the possibility that there is a negative feedback relationship between CS and plasma ionic calcium. Our data agree with this assumption.

Our observations show that the increase in CS size at high water-calcium levels is mainly or only caused by increase in size and, apparently, number of the type-1 cells. There is good evidence now, from different species of fish, that this cell type is the source of the hypocalcemic hormone of the CS (Wendelaar Bonga et al. 1976, 1980; Urasa and Wendelaar Bonga 1985). Changes in the calcium concentration of the food does not affect the plasma calcium level, or CS size and ultrastructure. This is in line with the conclusion of Flik et al. (1985a) that the water is the main calcium source, and that the gills are the main route of calcium uptake in *O. mossambicus*.

There is no evidence for involvement of the type-2 cells in calcium metabolism. CS and phosphate. The hypophosphatemia that occurs after removal of the CS may indicate that the CS produce a factor with a hyperphosphatemic action. In this case, exposure of fish to water with high phosphate levels might be expected to reduce the size of the CS as a result of inactivation of one or both of the endocrine cell types of the CS. No changes in the CSI, nor in the ultrastructure of the endocrine cell types of the CS are found, although plasma phosphate levels are elevated in fish from high-phosphate water. In our laboratory, it has been shown that phosphate enters *O. mossambicus* mainly via the gut. No evidence for branchial uptake is found (Flik et al. 1985b). This implies that phosphate from the water is taken up via drinking. Calculations based on the assumption that drinking rates of *O. mossambicus* in high-phosphate water are the same as under control conditions (Flik et al. 1985a) indicate that total phosphate uptake via drinking in fish in water containing 5 mM phosphate is 300% higher than in the controls.

Phosphate-deficient food has no noticeable effects on the size of the CS or on the ultrastructure of their endocrine cells. If the CS were involved in hyperphosphatemic control, activation of the CS might be expected, because the phosphate-deficient diet leads to hypophosphatemia. The diet has more severe effects on phosphate metabolism than is reflected by the relatively modest decrease in plasma phosphate. We have reported that it leads to considerable mobilization of phosphate from the skeleton and the scales (Urasa et al. 1985). Lopez (1970) has reported that, four weeks after the removal of the CS of European eels, the osteoclastic activity of the bone is reduced and the osteoclasts are almost absent. This observation indicates that in eels the CS maintain osteoclastic activity and thus phosphate release from the bone. In *O. mossambicus*, typical multinucleated osteoclasts have not been observed so far. The phosphate mobilization from bones and scales seems to be an acellular event. Apparently, the CS are not involved in the control of this process. High-phosphate food, although leading to significant hyperphosphatemia, does not influence CS size or ultrastructure. This is further evidence against a relationship between plasma phosphate and the CS.

We conclude that there is no evidence for direct involvement of the CS in the control of plasma phosphate levels or of phosphate metabolism in general. Unlike the plasma calcium concentration, the plasma phosphate level apparently is not a major factor for the control of CS activity. Whether the hypophosphatemia observed after removal of the CS has an endocrine basis remains unknown. Chan and Chester Jones (1968) have shown that, as a result of the combination of hypercalcemia with hypophosphatemia,

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**Table 3.** CSI and plasma total and ultrafiltrable calcium and phosphate concentrations of male *O. mossambicus* fed on a diet of artificial food with different calcium or phosphate concentrations for 6 weeks; *n* = 8

<table>
<thead>
<tr>
<th></th>
<th>CSI</th>
<th>Total Calcium</th>
<th>Total Phosphate</th>
<th>Ultrafiltrable Calcium</th>
<th>Ultrafiltrable Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.68 ± 0.08</td>
<td>3.01 ± 0.15</td>
<td>2.04 ± 0.13</td>
<td>1.48 ± 0.11</td>
<td>1.02 ± 0.11</td>
</tr>
<tr>
<td>Calcium-enriched</td>
<td>0.71 ± 0.11</td>
<td>2.96 ± 0.16</td>
<td>1.95 ± 0.14</td>
<td>1.51 ± 0.09</td>
<td>0.96 ± 0.11</td>
</tr>
<tr>
<td>Calcium-deficient</td>
<td>0.64 ± 0.07</td>
<td>2.90 ± 0.13</td>
<td>2.05 ± 0.13</td>
<td>1.60 ± 0.14</td>
<td>0.98 ± 0.16</td>
</tr>
<tr>
<td>Phosphate-enriched</td>
<td>0.74 ± 0.09</td>
<td>2.85 ± 0.14</td>
<td>2.43 ± 0.16*</td>
<td>1.41 ± 0.09</td>
<td>1.13 ± 0.08</td>
</tr>
<tr>
<td>Phosphate-deficient</td>
<td>0.72 ± 0.10</td>
<td>3.11 ± 0.17</td>
<td>1.85 ± 0.14**</td>
<td>1.46 ± 0.10</td>
<td>0.92 ± 0.17</td>
</tr>
</tbody>
</table>

* Significantly different from control, *P* < 0.05
** Significantly different from phosphate-enriched, *P* < 0.01
the molar concentration product of ultrafiltrable calcium and phosphate remained within normal values in stannicetomized eels. The mechanism responsible for this constancy is unclear.

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