Effect of Synthetic Salmon Calcitonin and Low Ambient Calcium on Plasma Calcium, Ultimobranchial Cells, Stannius Bodies, and Prolactin Cells in the Teleost Gasterosteus aculeatus

S. E. Wendelaar Bonga

University of Nijmegen, Department of Zoology, Nijmegen, The Netherlands

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Four daily injections of synthetic salmon calcitonin did not modify plasma calcium in seawater sticklebacks. The absence of a hypocalcemic response may be due to changes in the release of several hormones. Injection of calcitonin led to a reduction in the activity of the ultimobranchial cells and the type 1 cells of the Stannius bodies, as judged by ultrastructural examination and light microscopical morphometry. The prolactin cells became greatly stimulated. Since the Stannius type I cells likely produce a hypocalcemic hormone, and prolactin has a hypercalcemic action in this species, the observed changes may obscure any hypocalcemic effect of exogenous calcitonin. Thus, the absence of a hypocalcemic response to calcitonin does not exclude that the hormone has a hypocalcemic action. Such an action is indicated, since the responses of the three endocrine cell types to calcitonin were similar to the changes following exposure to low-calcium seawater.

Calcitonin has been isolated from several teleost species, and the role of the ultimobranchial bodies as the production site of this hormone in fish is well established (Orimo et al., 1972; Tisserand-Jochem et al., 1977). Calcitonin isolated from salmon or eel shows a high specific hypocalcemic activity, at least in rat and mouse bioassays (Orimo et al., 1972; Keutmann et al., 1978). Nevertheless, the physiological role of calcitonin in teleosts is still in discussion. Many authors have been unable to demonstrate an effect of calcitonin on plasma calcium levels in fish (Pang and Pickford, 1967; Pang, 1971; Copp et al., 1972; Suryawanshi and Mahajan, 1976). Only a few experiments have been reported showing that mammalian or fish calcitonin lowers plasma calcium (Chan et al., 1968; Pang, 1971; Lopez et al., 1976). The many negative results have led to the supposition that in teleosts calcitonin is not specifically involved in the control of calcium metabolism (Pang, 1971; Suryawanshi and Mahajan, 1976; Yamauchi et al., 1978).

However, calcitonin has recently been shown to enhance renal calcium excretion (Fenwick, 1978), to stimulate branchial calcium outflux, and to reduce branchial calcium influx (Milhaud et al., 1977; Peignoux-Deville et al., 1978). Therefore, the absence of a hypocalcemic response after calcitonin administration may be related to the experimental design, rather than to the absence of a hypocalcemic action of the hormone. Since plasma calcium is controlled within relatively narrow limits in many fish species, a hypocalcemic action of injected calcitonin may be obscured by changes in the release of endogenous calcitonin and of other calcium regulating hormones. In this respect the hypocalcemic factor of the Stannius bodies (hypocalcin) and prolactin, that likely has hypercalcemic capacities in fish (Pang et al., 1973; Olivereau and Olivereau, 1978; Wendelaar Bonga et al., 1978), are of interest.

To investigate whether exogenous calcitonin affects the release of endogenous calcitonin and of other hormones, a structural analysis was performed of the cells producing calcitonin, hypocalcin, and prolactin. These cells were examined ultrastructurally and by light microscopical
morphometry. The effects of calcitonin injections were compared to the changes induced in the same cell types by exposure of fish to low-calcium seawater. This treatment imposes hypocalcemic stress on the animals.

MATERIALS AND METHODS

Adult, sexually immature, female sticklebacks (65–70 mm in body length) were obtained from the Wadden Sea in early spring. They were kept for at least 4 weeks in artificial seawater (10.2 mmol Ca\(^2+\)/liter), at 15° and 8 hr of light per day.

(a) Calcitonin injection. Each of a group of seawater fish received four daily injections of synthetic salmon calcitonin (Calbiochem), dissolved in 10 \(\mu\)l of 0.6% saline containing 1% gelatin. Controls were injected with solvent only. Three hours after the last injection the fish were killed.

(b) Low-calcium exposure. Seawater sticklebacks were exposed for 4 days to Hale’s artificial seawater containing 0.8 mmol Ca\(^2+\)/liter. The osmolarity of this low-calcium solution was adjusted with NaCl to that of the control solution, Hale’s seawater (10.2 mmol Ca\(^2+\)/liter; for composition see Wendelaar Bonga, 1978).

After anesthetization of the fish with MS 222 (Sandoz), blood samples were collected from the caudal artery. Total plasma calcium was determined with a Marius calcium titrator. The ultimobranchial bodies, the Stannius bodies, and the pituitary glands were prepared for light and electron microscopy.

For light microscopy, paraffin sections of the Bouin-fixed endocrine glands were stained with Mayer’s hematoxylin and eosin. The volumes of cells and nuclei were determined as described earlier (Wendelaar Bonga el al., 1976). For electron microscopy the glands were prefixed in cacodylate-buffered (0.1 M, pH 7.4) 3% glutaraldehyde solution for 15 min at room temperature. They were fixed in a similarly buffered solution of 1% osmium tetroxide, 1.5% glutaraldehyde, and 2.5% potassium dichromate, for 1 hr at 0°. They were postfixed in 1% uranyl acetate and embedded in Spurr’s resin or Epon.

RESULTS

Plasma Calcium

(a) Calcitonin injection. After four daily injections of synthetic salmon calcitonin (10 mU/g), total plasma calcium concentration was unchanged (6.0 ± 0.4; controls, 5.7 ± 0.5 mmol/liter). In a similar experiment, not reported here, doses of 50 mU/g were equally ineffective.

(b) Low-calcium exposure. Four days after transfer of seawater sticklebacks to low-calcium seawater, total plasma calcium was slightly, but not significantly, reduced (5.2 ± 0.4; controls, 5.9 ± 0.3 mmol/liter).

Ultimobranchial Cells

The ultimobranchial body of sticklebacks contains granular secretory cells and non-granular supporting cells. The supporting cells separate the secretory cells over large areas from the surrounding connective tissue. The cytoplasm of the secretory cells contains strands of granular endoplasmic reticulum, many free ribosomes, some small mitochondria, a Golgi field, and dense-cored secretory granules with a diameter of about 110 nm (Fig. 1).

(a) Calcitonin injection. The ultimobranchial cells of control fish were well developed and secretory active. The Golgi areas were extensive and often contained electron-dense presecretory material. Signs of release of secretory material by exocytosis were occasionally observed. In the calcitonin-treated fish the ultimobranchial cells were apparently less active than in the control fish. The cells and their nuclei were considerably smaller (Table 1). Golgi areas were insignificant and did not contain presecretory material. The number and size of the lysosomes had markedly increased. Intracellular digestion of cytoplasmic material, including secretory granules, was indicated in many cells by the presence of many large autophagosomes (Fig. 2). In such cells the membranes of the endoplasmic reticulum were often dilated, forming vacuolar structures. In many other cells secretory granules were present in large numbers, which points to reduced hormone release (Fig. 3). Indications for exocytosis were not found.

(b) Low-calcium exposure. Transfer of sticklebacks to low-calcium seawater induced the same changes in the ultimobranchial cells as calcitonin injection. The size of cells and nuclei decreased significantly in comparison to the controls (Table 1). Signs of formation or release of secretory granules were no longer observed. Autophagosomes were even more frequent...
Fig. 1. Ultimobranchial cells of a seawater fish (control) showing small electron-dense secretory granules, strands of granular endoplasmic reticulum, numerous free ribosomes, and a large Golgi area (Ga). ngc, nongranular cell; ct, connective tissue.

Fig. 2. Cytoplasm of an ultimobranchial cell of a calcitonin-injected seawater fish showing two autophagosomes (arrows) containing secretory granules and ribosomes.

Fig. 3. Ultimobranchial cell of a calcitonin-injected seawater fish; the cytoplasm contains many secretory granules.
TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>UBL nuclei</th>
<th>Stannius bodies</th>
<th>Prolactin cells</th>
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<tr>
<td></td>
<td></td>
<td>Nuclei type 1</td>
<td>Nuclei type 2</td>
</tr>
<tr>
<td>(a) Controls</td>
<td>31.4 ± 3.1</td>
<td>35.3 ± 3.6</td>
<td>29.2 ± 4.5</td>
</tr>
<tr>
<td>4 × SSC</td>
<td>18.3 ± 3.0**</td>
<td>25.8 ± 2.2**</td>
<td>25.5 ± 3.5</td>
</tr>
<tr>
<td>(b) Controls</td>
<td>34.7 ± 4.7</td>
<td>34.8 ± 2.8</td>
<td>26.6 ± 3.8</td>
</tr>
<tr>
<td>Low Ca²⁺</td>
<td>20.8 ± 3.6**</td>
<td>27.6 ± 2.9**</td>
<td>28.5 ± 4.1</td>
</tr>
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* Significantly different from controls, \( P < 0.05 \).
** Idem, \( P < 0.01 \).

than after calcitonin treatment. The cells containing such bodies often showed vacuolization of the cytoplasmic matrix, the endoplasmic reticulum, and the mitochondria (Fig. 4). However, in many other cells such signs of cellular breakdown were absent (Fig. 5). Occasionally macrophages were present between the ultimobranchial cells.

**Stannius Bodies**

( a ) Calcitonin injection. The ultrastructure of the two endocrine cell types of the Stannius bodies of sticklebacks has been described earlier (Wendelaar Bonga et al., 1976).

In the control fish the type 1 cells, characterized by large secretory granules, were well developed. The cytoplasm contained large arrays of granular endoplasmic reticulum. In the extensive Golgi fields, presecretory granules, likely originating by budding from the Golgi membranes, were common (Fig. 6). Indications of exocytosis were occasionally observed.

In the calcitonin-treated fish the type 1 cells were smaller. The volume of their nuclei was significantly reduced (Table 1). The extent of the granular endoplasmic reticulum and the Golgi fields had diminished. Indications of formation of secretory granules were only incidentally found, while exocytosis was not observed. The number of secretory granules in the cytoplasm had increased (Fig. 7).

The type 2 cells, small and inactive in seawater fish (Wendelaar Bonga et al., 1976), were not noticeably influenced by calcitonin. The volume of the nuclei was unchanged (Table 1).

(b) Low-calcium exposure. After low-calcium exposure the type 1 cells showed similar signs of reduced secretory activity as after calcitonin treatment. The size of cells and nuclei (Table 1) and the extent of granular endoplasmic reticulum and Golgi system had decreased almost to the same extent. No changes were observed in the type 2 cells.

**Prolactin Cells**

( a ) Calcitonin injection. In seawater control sticklebacks the prolactin cells were small and inactive (Fig. 8), as has been described earlier (Wendelaar Bonga, 1978). After calcitonin treatment the structure of the cells had changed markedly. Cell and nuclear volume had increased significantly (Table 1). The extent of the granular endoplasmic reticulum and of the Golgi areas, small in cells of control fish, had enlarged. In the Golgi areas signs of active secretion, such as the presence of electron-dense material in the Golgi saccules and of presecretory granules in the surrounding area, were commonly observed (Fig. 9).

(b) Low-calcium exposure. The changes that occurred after transfer to low-calcium seawater were similar to those of calcitonin treatment, as far as cell and nuclear vol-
Fig. 4. Ultimobranchial cell of a fish exposed to low-calcium seawater: the cytoplasm shows autophagous vacuoles (arrows) containing mitochondria, secretory granules, and ribosomes: the granular endoplasmic reticulum is vacuolated (v).

Fig. 5. Ultimobranchial cell of a fish exposed to low-calcium seawater: the cytoplasm contains many secretory granules.

DISCUSSION

Prolonged injection of calcitonin did not noticeably influence total plasma calcium in seawater sticklebacks. A hypocalcemic response after calcitonin injection has been reported for European and American eels (Chan et al., 1968; Pang, 1971; Peignoux-Deville et al., 1975). Other positive results concern the catfish Ictalurus melas (Louw et al., 1967) and the trout Salmo gairdnerii (Lopez et al., 1971).

Many attempts to influence plasma calcium by administration of calcitonin have been unsuccessful (Pang and Pickford, 1967; Pang, 1971; Copp et al., 1972; Suryawanshi and Mahajan, 1976; Milhaud et al., 1977; Dacke, 1972; Yamauchi et al., 1978). In some experiments changes in plasma chloride or osmolarity have been observed, and it has thus been suggested that teleostean calcitonin, rather than being involved in calcium metabolism, is primarily concerned with hydromineral regulation in general (Pang, 1971; Orimo et al., 1972; Suryawanshi and Mahajan, 1976; Yamauchi et al., 1978). The present results on sticklebacks indicate that this suggestion is premature. A possible hypocalcemic action of exogenous calcitonin will be counteracted by changes occurring in the release of hormones like calcitonin, hypocalcin, and prolactin. In the highly active ultimobranchial cells of seawater sticklebacks, indications of reduced secretory activity were overt after calcitonin treatment. The probable reduction of calcitonin release will reduce the effect of the exogenous hormone. Calcitonin also reduced the secretory ac-
Fig. 6. Stannius body of a seawater fish (control): type 1 cells containing large secretory granules, strands of rough endoplasmic reticulum, and a Golgi area with presecretory granules (arrows).

Fig. 7. Stannius corpuscle of a seawater fish injected with calcitonin. Large secretory granules are accumulated in the cytoplasm of the type 1 cells (t-1). t-2, type 2 cells.
Fig. 8. Prolactin cell of a seawater fish (control) with many secretory granules, a small Golgi area (Ga), and a few strands of endoplasmic reticulum. sc, stellate cells.

Fig. 9. Prolactin cells of a seawater fish injected with calcitonin, showing extensive Golgi areas (Ga) with presecretory granules (arrows). mvb, multivesicular body; sc, stellate cell.
Activity of the Stannius type 1 cells. The hypocalcemic factor produced by these cells likely reduces calcium uptake in the gills (Fenwick and So, 1974; Milet et al., 1975). The same effect has been described for calcitonin (Peignoux-Deville et al., 1978). Thus, the reduced secretory activity of the Stannius type 1 cells after calcitonin treatment will diminish the effect of the latter hormone.

The prolactin cells became considerably activated after four injections of calcitonin. There is growing evidence that in teleosts prolactin is involved in the endocrine control of calcium metabolism (Pang et al., 1973; Wendelaar Bonga and Greven, 1978). Prolactin has a hypercalcemic activity, as was demonstrated in killifish (Pang et al., 1973), eel (Olivereau and Olivereau, 1978), sticklebacks (Wendelaar Bonga et al., 1978), and the cichlid fish *Sarotherodon mossambicus* (Wendelaar Bonga and Van der Meij, 1979). The observed rise in prolactin cell activity after low-calcium exposure is in line with the hypercalcemic action of this hormone. The activation of the prolactin cells after calcitonin injection is likely accompanied by enhanced prolactin release. The hypercalcemic effect of this hormone will counteract any hypercalcemic effect of calcitonin.

On the basis of the above results we conclude that the absence of a noticeable hypocalcemia after calcitonin administration does not allow the conclusion that the hormone has no hypocalcemic action. Evidence in favor of a hypocalcemic action is provided by the results of low-calcium exposure. This treatment represents a hypocalcemic stress for the animals (Wendelaar Bonga et al., 1978). The changes induced by low-calcium exposure in the

![Fig. 10. Prolactin cell of a fish exposed to low-calcium seawater; the cytoplasm contains a large Golgi area with several presecretory granules (arrows).](image-url)
Stannius type 1 cells and the prolactin cells were similar to the effects of calcitonin injection on these cell types. This similarity points to calcitonin as a hypocalcemic factor. The observed reduction of the ultimobranchial cells in the low-calcium-adapted fish is in line with this conclusion.

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