Effect of Synthetic Salmon Calcitonin on Protein-Bound and Free Plasma Calcium in the Teleost Gasterosteus aculeatus

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A single injection of synthetic salmon calcitonin induced a transient decrease in the free-calcium fraction of blood plasma of freshwater sticklebacks. This decrease was especially pronounced in fish adapted to low-calcium freshwater. The protein-bound calcium fraction was not noticeably affected. The often reported absence of a hypocalcemic effect of calcitonin injections in fish may partly be attributed to the determination in most studies of total plasma calcium levels only, or to the transient nature of the hypocalcemic response.

Eel and salmon calcitonin have potent hypocalcemic activities in rat and mouse bioassays (Orimo et al., 1972; Keutmann et al., 1978). The question whether this hormone also influences blood calcium in teleost fish has not been answered satisfactorily up till now. Some authors showed that mammalian or fish calcitonin reduces total plasma calcium in fish (Louw et al., 1967; Chan et al., 1968; Lopez et al., 1971; Peignoux-Deville et al., 1975), but many attempts to demonstrate an effect of calcitonin on total plasma calcium remained unsuccessful (Pang and Pickford, 1967; Pang, 1971; Copp et al., 1972; Yamauchi et al., 1978a; Wendelaar Bonga, 1980). These findings have led some authors to conclude that in teleosts calcitonin is not specifically involved in the hormonal control of calcium metabolism, but rather in hydromineral regulation in general (Orimo et al., 1972; Suryawanshi and Mahajan, 1976), or in sexual maturation (Yamauchi et al., 1978b).

In this note the effects of synthetic salmon calcitonin on plasma ion composition and osmolarity in sticklebacks adapted to normal freshwater and to low-calcium freshwater are described. In fish from low-calcium freshwater the calcitonin production in the ultimobranchial body is likely to be very low (Wendelaar Bonga, 1980). This may facilitate the detection of any effect of the exogenous hormone. Evidence is presented that calcitonin reduces the free-calcium fraction in blood plasma.

MATERIALS AND METHODS

Adult sticklebacks (Gasterosteus aculeatus trachurus), 2.5-3.0 g body wt, were caught in canals in early spring. Since sexual maturation affects calcium metabolism in males as well as females, only immature fish were used. The data presented concern female fish with a gonadosomatic index varying between 0.052 and 0.061 (ratio of gonadal and body weight). They were kept for at least 4 weeks in freshwater aquaria at 22° under a daily light period of 8 hr. One week prior to injection fish were transferred to artificial freshwater (Ca**:0.80 mmol/liter; for composition see Wendelaar Bonga and Van der Meij, 1980) or to low-calcium artificial freshwater (Ca**:0.08 mmol/liter). Synthetic salmon calcitonin (specific activity 4000 U/mg; gift from Armour Pharmaceutical Company, Kankakee, Ill.) was administered by a single intraperitoneal injection of 10 ml/g, dissolved in 10 ml of a solution of 0.6% NaCl and 1% gelatin in distilled water. Controls were injected with a similar volume of the solvent. Six hours after injection (freshwater fish) or 2, 6, and 10 hr after injection (low-calcium-adapted fish) animals were anesthetized in a solution of MS-222. Blood was collected from the caudal arteries. Plasma samples of four fish were pooled and one part of the plasma was used for determination of total plasma calcium, Na*, K+, Cl-, and osmolarity. The other part was deproteinized by ultrafiltration (Sartorius Membran filter, cut off: 1000 M), and free-plasma calcium was determined in the filtrate. The techniques used were reported earlier (Wendelaar Bonga and Van der Meij, 1980). Results were tested for significance by Student's t test (two tailed).
RESULTS

Six hours after injection of calcitonin in freshwater sticklebacks total plasma calcium did not differ significantly from that of controls. However, free-calcium concentration was 24% lower than the control value (Table 1).

In calcitonin-treated fish adapted to low-calcium freshwater, total plasma calcium as well as the free-calcium fraction were significantly lower than those in control fish (Table 1). Data show that almost 90% of the reduction in the total plasma calcium concentration was accounted for by the decrease in the free-calcium fraction. This decrease of free-calcium concentration appeared transient, as in blood plasma sampled 2 or 10 hr after calcitonin injection free-calcium levels were similar to those of control fish (Fig. 1).

In both experimental groups plasma Na⁺, Cl⁻, and osmolarity were unaffected by exogenous calcitonin. Plasma K⁺ concentration, unchanged in freshwater fish, was significantly enhanced in the calcitonin-treated low-calcium-adapted fish (Table 1).

DISCUSSION

Synthetic salmon calcitonin reduces the free-calcium fraction in the blood plasma of sticklebacks. In fish, as in mammals, this fraction is composed of ionic calcium and of calcium complexed with phosphate, citrate, or carbonate (Chan and Chester Jones, 1968). Because of the constancy of the ratio

![Figure 1. Total and free-plasma calcium of fish from low-calcium freshwater (means ± SD of six samples, each sample containing blood from four fish), after a single injection (at 0 hr) of synthetic salmon calcitonin (solid lines) or solvent (dotted lines).](image)

### TABLE 1

**Total Calcium, Free Calcium, Na⁺, K⁺ and Cl⁻ Concentrations, and Osmolarity in Blood Plasma of Fish from Freshwater and Low-Calciuam Freshwater (Means ± SD of Six Samples, Each Sample Containing Plasma of Four Fish), 6 hr after Injection of Synthetic Salmon Calcitonin or Solvent (Controls)**

<table>
<thead>
<tr>
<th></th>
<th>Freshwater Controls</th>
<th>Freshwater Calcitonin</th>
<th>Low-Ca²⁺ freshwater Controls</th>
<th>Low-Ca²⁺ freshwater Calcitonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total calcium (meq/liter)</td>
<td>5.6 ± 0.4</td>
<td>5.1 ± 0.3</td>
<td>5.2 ± 0.4</td>
<td>4.3 ± 0.3*</td>
</tr>
<tr>
<td>Free calcium (meq/liter)</td>
<td>2.9 ± 0.3</td>
<td>2.2 ± 0.2**</td>
<td>2.4 ± 0.2</td>
<td>1.6 ± 0.2**</td>
</tr>
<tr>
<td>Na⁺ (meq/liter)</td>
<td>145 ± 3</td>
<td>143 ± 6</td>
<td>136 ± 4</td>
<td>128 ± 5</td>
</tr>
<tr>
<td>K⁺ (meq/liter)</td>
<td>6.4 ± 0.7</td>
<td>7.2 ± 0.8</td>
<td>7.6 ± 0.5</td>
<td>10.8 ± 0.6**</td>
</tr>
<tr>
<td>Cl⁻ (meq/liter)</td>
<td>118 ± 5</td>
<td>115 ± 6</td>
<td>117 ± 3</td>
<td>119 ± 4</td>
</tr>
<tr>
<td>Osmolarity (mosm/liter)</td>
<td>308 ± 4</td>
<td>313 ± 7</td>
<td>298 ± 6</td>
<td>289 ± 5</td>
</tr>
</tbody>
</table>

* Significantly different from controls, \( P < 0.05 \).

** idem, \( P < 0.01 \).
between the ionic and complexed calcium concentrations (Chan and Chester Jones, 1968), calcitonin likely affects the physiologically important plasma calcium fraction.

The effect of calcitonin on plasma calcium was more pronounced in fish adapted to low-calcium freshwater than in fish from normal freshwater. In the latter fish total plasma calcium was not significantly reduced, possibly due to a masking effect of the large protein-bound calcium fraction. In the low-calcium-adapted fish the reduction of the free calcium concentration accounted for most of the decrease in total plasma calcium. No clear evidence could be found for an effect of calcitonin on the protein-bound fraction.

Our data show that the effect of a single injection of calcitonin on plasma calcium in sticklebacks is of short duration. A similar observation was made by Chan et al. (1968) in eel. This may be due to rapid metabolism of the hormone after injection. The possibility cannot be excluded that the hypocalcemic effect is counteracted by hypercalcemic factors. In this respect prolactin needs attention, since this hormone enhances total plasma calcium in fish (Pang et al., 1971; Copp et al., 1972; Orimo et al., 1972; Yamauchi et al., 1978; Wendelaar Bonga, 1980) may have several causes. It may partly be due to the fact that in most studies only total plasma calcium was determined. An effect of the injected hormone may have been masked by the protein-bound calcium, as was similar in the freshwater fish examined in this study. However, other factors are likely involved in addition. Calcitonin may have been administered in inappropriate concentrations, or the effect of the hormone may have escaped attention because of its short duration. Our preliminary experiments on male and female immature sticklebacks have shown that the time lag between calcitonin injection and hypocalcemic response is highly temperature dependent. In fish adapted to 15, instead of 22° in the present experiments, a significant hypocalcemia did not occur before 9 hr after hormone administration. Finally, the possibility needs consideration that there are species-specific differences in the responses to exogenous calcitonin.

Calcitonin did not noticeably influence plasma Na⁺, Cl⁻, or osmolarity. Changes in plasma Cl⁻ (Pang, 1971) and osmolarity (Orimo et al., 1972) have been reported after calcitonin injection in fish. We did find a significant increase in the plasma K⁺ concentration of the low-calcium-adapted fish, although this rise may have been caused by leakage of K⁺ from the blood cells. A reduction in plasma ionic calcium is known to enhance the permeability of cellular membranes for K⁺ (Morrill and Robbins, 1967).

The failure to demonstrate an effect of calcitonin on plasma calcium in fish (Pang et al., 1971; Copp et al., 1972; Orimo et al., 1972; Yamauchi et al., 1978a; Wendelaar Bonga, 1980) may have several causes. It may partly be due to the fact that in most studies only total plasma calcium was determined. An effect of the injected hormone may have been masked by the protein-bound calcium, as was similar in the freshwater fish examined in this study. However, other factors are likely involved in addition. Calcitonin may have been administered in inappropriate concentrations, or the effect of the hormone may have escaped attention because of its short duration. Our preliminary experiments on male and female immature sticklebacks have shown that the time lag between calcitonin injection and hypocalcemic response is highly temperature dependent. In fish adapted to 15, instead of 22° in the present experiments, a significant hypocalcemia did not occur before 9 hr after hormone administration. Finally, the possibility needs consideration that there are species-specific differences in the responses to exogenous calcitonin.

REFERENCES


