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Hypocalcemic Effects of Bovine Parathyroid Hormone (1–34) and Stannius Corpuscle Homogenates in Teleost Fish Adapted to Low-Calcium Water

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ABSTRACT

Injections of bovine parathyroid hormone (PTH 1–34) and homogenates of corpuscles of Stannius produce hypocalcemia in male killifish and tilapia adapted to calcium-deficient seawater or fresh water, respectively. In fish from water with normal calcium concentrations no effects are noticeable. These results suggest similarity in bioactivity between PTH, the hypercalcemic hormone of terrestrial vertebrates, and the hypocalcemic factor of the corpuscles of Stannius in teleost fish.

Typical parathyroid glands (PT) have not been described for fish and seem to be confined to the terrestrial vertebrates (Pang, '73; Roth and Schiller, '76). It has been suggested that the corpuscles of Stannius (CS), small endocrine glands in the kidneys of holostean and teleostean fish, are homologous with the PT glands (Milet et al., '79a, '80; Lopez et al., '84). This suggestion was mainly based on the immunological resemblance and similarities in bioactivity of parathyroid hormone (PTH) and substances present in the CS. Although true homology between PT and CS has been questioned (Orimo et al., '82; Wendeelaar Bonga and Pang, '86), there are undeniably striking similarities between the effects of PTH and CS homogenates. Milet et al. ('79a) have reported that CS homogenates increase calcium release from rat tibial bone in vitro, similar to PTH. Recently, we have confirmed this observation for mouse calvaria in vitro. We have further shown that in this preparation CS extracts have a comparable action to PTH on phosphate release and lactate production (Lafeber et al., '84, '86).

With respect to the effects on blood calcium concentrations, comparison of PTH and CS homogenates has produced confusing results. In mammals, birds, reptiles, and most amphibians, PTH is generally considered a potent hypercalcemic hormone (Dacke and Kenny, '73; Pang et al., '80; Sasayama and Oguro, '82). Reports on the effects of CS extracts on plasma calcium levels in tetrapods are conflicting: hypercalcemic as well as hypocalcemic actions have been reported for rats (Leung and Fenwick, '78; Milet et al., '79a). CS homogenates induced hypocalcemia in birds (Srivastav and Swarup, '82), whereas for amphibians hypocalcemia (Pandey et al., '82; Milet et al., '84) as well as hypercalcemia have been reported (Milet et al., '84).

In teleost fish, the hypocalcemic function of the CS has been well established (Fenwick, '82). However, the effects of PTH in fish are not clear. Increased (Fleming and Meier, '61a) as well as decreased calcium levels (Clark and Fleming, '63; Milet et al., '85) have been reported after PTH treatment of fish. In these experiments, the fish had been made hypercalcemic before injection by estrogen or by removal of the CS, and thus the effects were antihypercalcemic rather than hypocalcemic. In intact fish with normal calcium levels no effects of PTH could be found (Fleming and Meier, '61b; Moss, '63; Pang, '73). This is not surprising since CS extracts also appear to have no substantial effect on plasma calcium when injected into intact fish maintained in water with calcium levels in the normal range (Pang et al., '73; Fenwick, '82). In contrast, significant hypocalcemia could be induced by CS extracts in killifish from calcium-deficient seawater (Pang et al., '74). In this study, the authors assumed that fish from calcium-deficient water have low circulating levels of endogenous hormones and are therefore more responsive to exogenous hypocalcemic factors. In the present paper we therefore compared the effects of syn-
thetic bovine PTH (1–34) and CS homogenates on plasma calcium levels in two species of teleost fish adapted to low-calcium water.

MATERIALS AND METHODS

Animals

Male *Fundulus heteroclitus* were purchased from a commercial supplier in New York City. The animals were kept at 15°C in artificial calcium-deficient seawater (Ca$^{2+}$: between 0.1 and 0.05 mM) and fed low-calcium food, as described previously (Pang et al., '73). They were kept under these conditions for at least 6 weeks before use in Lubbock, Texas. Male tilapia (*Oreochromis mossambicus*; formerly *Sarotherodon mossambicus*) were reared in the laboratory at Nijmegen at 25°C in tap water (Ca$^{2+}$: 0.8 mM). Four to five weeks before the experiments the fish were transferred to artificial low-calcium fresh water. The Ca$^{2+}$ concentration was reduced stepwise during the first week from 0.8 mM to 0.1 mM. For the adaptation protocol and the formula of the artificial fresh water (see Wendelaar Bonga and Van der Meij ('80)).

CS homogenates were prepared from the CS of freshwater rainbow trout of both sexes. After anesthetizing the fish with MS 222, the glands were removed. The CS were frozen on dry ice for less than a week and injected after homogenization in saline (50 mg CS in 1 ml 0.6% NaCl). Tilapia CS homogenates were prepared from CS removed from male or female tilapia of about 20 g body weight and were adapted for 4 weeks to high-calcium tap water (5 mM CaCl$_2$ in tap water). This treatment increased the size of the glands and their hormone content (Urasa and Wendelaar Bonga, '86). After dissection the CS were kept on ice, homogenized in saline (50 mg CS in 1 ml 0.6% NaCl), and injected the same day. At the time of use, the body weight of the injected tilapia amounted to 10.3 ± 0.4 g wet weight (n = 67).

Injections

Killifish received four daily intraperitoneal injections (volume: 10 µl per g body weight) of either trout CS homogenate, synthetic bovine PTH (1–34), or solvent. The bPTH was obtained from Bachem (Bubendorf, Switzerland) and dissolved in 0.005 M acetic acid with 0.1% albumin. To test the effect of different hormone concentrations, fish were bled 2 hours after the last daily injection on the fourth day (killifish) or 90 minutes after a single injection (tilapia). Preparation of blood plasma and determination of plasma total calcium levels were the same as reported previously for killifish (Pang et al., '73) and tilapia (Wendelaar Bonga et al., '83), respectively. To analyse the time course of the effect of bPTH and CS homogenates, blood was collected 30, 60, 90, and 120 minutes after a single injection of bPTH or CS homogenates in tilapia. For every time period a different group of fish was used. Blood plasma was collected after centrifugation, and plasma total calcium was determined by atomic absorption spectrophotometry in the presence of lanthanum.

Statistics

The data were analysed for statistical significance by Student's t-test.

RESULTS

Killifish

After 2 hours, injections of trout CS homogenate induce a significant reduction of plasma total calcium in killifish adapted to calcium-deficient seawater. Also bPTH (1–34) decreases plasma total calcium. The reduction is statistically significant with doses of 0.01 µg·g$^{-1}$ or higher (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Calcium level</th>
<th>Dose (µg·g$^{-1}$)</th>
<th>Plasma total calcium (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Low</td>
<td>500</td>
<td>2.89 ± 0.04 (16)</td>
</tr>
<tr>
<td>CS</td>
<td>Low</td>
<td>0.001</td>
<td>2.79 ± 0.05 (8)*</td>
</tr>
<tr>
<td>bPTH (1–34)</td>
<td>Low</td>
<td>0.003</td>
<td>2.72 ± 0.09 (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01</td>
<td>2.54 ± 0.07 (16)**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03</td>
<td>2.52 ± 0.10 (8)**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>2.53 ± 0.07 (8)**</td>
</tr>
<tr>
<td>Control</td>
<td>Normal</td>
<td>–</td>
<td>2.80 ± 0.02 (7)</td>
</tr>
<tr>
<td>CS</td>
<td>Normal</td>
<td>700</td>
<td>2.79 ± 0.12 (7)</td>
</tr>
</tbody>
</table>

*Blood plasma was collected 2 hours after the last injection. Means ± SD. The number of fish per group is indicated between brackets.

*Significantly different from the control group (P < 0.05).

**Significantly different from the control group (P < 0.01).
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A single injection of 0.1 µg·g⁻¹ bPTH (1–34) in male tilapia adapted to low-calcium fresh water produces a reduction of plasma total calcium that becomes noticeable after 60 minutes and is statistically significant below the control values after 90 minutes and 120 minutes. Injection of 150 µg·g⁻¹ of CS homogenates has similar effects (Fig. 1). Tilapia adapted to low-calcium fresh water show a statistically significant reduction of plasma total calcium 90 minutes after a single injection of tilapia CS homogenates. Plasma total calcium is also reduced after injection of bPTH (1–34) in doses of 0.05 or 0.10 µg·g⁻¹ (Table 2). The reduction of plasma total calcium is log-dose related. No effect of bPTH (1–34) is noticeable after injection in fish adapted to fresh water with normal Ca²⁺ levels (Table 2).

DISCUSSION

Injections of bPTH (1–34) produce hypocalcemia in both species examined, as do injections of CS homogenates. This is the first time that true PTH-induced hypocalcemia is observed in fish. Reduction of plasma calcium levels by PTH or PT extracts has so far only been reported for estrogen-treated killifish (Clark and Fleming, '63) and stannietctomized eels (Milet et al., '85). Estrogen treatment and staniectomy both lead to marked elevation of the plasma calcium levels, in particular by increasing the protein-bound calcium fraction. Injections of PTH reduced or prevented the hypercalcemia that normally follows these treatments, without inducing true hypocalcemia. In otherwise untreated fish no changes in plasma calcium could be observed with PTH (Fleming and Meier, '61a; Moss, '63; Pang, '73).

The positive results we obtained for intact male killifish and tilapia are apparently facilitated by the low calcium concentrations of the ambient water used in our study. Adaptation of fish to low-calcium water will lead to inactivation of the CS and of the ultimobranchial bodies (Wendelaar Bonga et al., '76; Urasa and Wendelaar Bonga, '86). These glands are the sources of the two principal hypocalcemic factors in fish: the putative hormone of the CS, called hypocalcin (Pang et al., '74) or teleocalcin (Ma and Copp, '78), and calcitonin, the hormone of the ultimobranchial bodies. The use of fish from low-calcium water in our study was based on the assumption that the effect of an exogenous hypocalcemic hormone will be more easily detected when the circulating levels of the endogenous hypocalcemic hormones are reduced (Pang et al., '74). For evoking even a minor response to CS homogenates in killi-

**Fig. 1. Effects of a single injection of 0.1 µg·g⁻¹ bPTH (1–34) (open bars) and of 150 µg·g⁻¹ tilapia CS homogenate (dark bars) on plasma total calcium levels of male tilapia adapted to low-calcium fresh water. Blood was collected 30 minutes (n = 4), 60 minutes (n = 4), 90 minutes (n = 8), or 120 minutes (n = 4) after injection; ctr, control values obtained 90 minutes after injection with solvent, (means ± S.D.).*, Significantly different from controls (P < 0.05); **, idem (P < 0.01).**

**TABLE 2. Effects of a single injection of bPTH (1–34) or tilapia CS homogenate on plasma calcium levels of male tilapia adapted to low-calcium fresh water (low Ca; 0.1 mM Ca) or fresh water with normal calcium levels (normal Ca; 0.8 mM Ca).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Calcium level</th>
<th>Dose (µg·g⁻¹)</th>
<th>Plasma total calcium (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Low</td>
<td>0.005</td>
<td>2.57 ± 0.10</td>
</tr>
<tr>
<td>Tilapia CS</td>
<td>Low</td>
<td>150</td>
<td>2.27 ± 0.14 (8)*</td>
</tr>
<tr>
<td>bPTH(1–34)</td>
<td>Low</td>
<td>0.06</td>
<td>2.39 ± 0.09 (8)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.10</td>
<td>2.23 ± 0.08 (8)**</td>
</tr>
<tr>
<td>Control</td>
<td>Normal</td>
<td>0.10</td>
<td>2.84 ± 0.17 (8)</td>
</tr>
<tr>
<td>Tilapia CS</td>
<td>Normal</td>
<td>150</td>
<td>2.95 ± 0.21 (6)</td>
</tr>
<tr>
<td>bPTH (1–34)</td>
<td>Normal</td>
<td>0.005</td>
<td>2.63 ± 0.11 (10)</td>
</tr>
</tbody>
</table>

*Plasma was collected 90 minutes after injection. Means SD. The number of fish per group is indicated between brackets.

*Significantly different from the control group (P < 0.05).

**Significantly different from the control group (P < 0.01).
fish from seawater with a normal calcium level (10 mM), large amounts of CS tissue are required (Pang et al., '74). The present data on tilapia show that a dose of CS extract sufficient to produce a significant hypocalcemia in fish from low-calcium water is ineffective in fish from water with normal calcium levels. Our data seem to indicate that the hypocalcemic potency of bPTH (1–34) in killifish is higher, by a factor of ten, than in tilapia. This difference may, however, be related to the difference in the experimental protocols, as the killifish received four daily injections and the tilapia a single injection. Moreover, the calcium content of food and water was substantially lower for killifish than for tilapia. The PTH-induced hypocalcemia develops rapidly after injection, as it does after injection of CS homogenates. A prompt response to CS homogenates has been reported before for killifish and eels (Pang et al., '74; Bailey and Fenwick, '75). The hypocalcemia may be caused by changes in calcium fluxes over the gills. Removal of the CS of eels leads to an increase of branchial calcium influx (So and Fenwick, '77; Milet et al., '79b). PTH as well as CS extracts affect a decrease in influx and an increase in outflux of calcium in perfused gill arches of eels (Milet et al., '79a, '80) 10 to 30 minutes after the start of the perfusion.

Our results confirm that there are striking similarities in the biological action of PTH and the substances produced by the CS. Apart from the reported stimulation of resorption of mammalian bone in vitro (Milet et al., '79b; Lafeber et al., '84, '86), PTH and CS have in common the ability to stimulate $^{45}$Ca influx and inhibit $^{45}$Ca outflux in an isolated eel gill preparation (Milet et al., '85). In mouse calvarial bone, both substances probably act via the same receptor (Lafeber et al., '86). In combination with the immunological cross-reactivity observed in eel CS after staining with antibodies against PTH and the slight ultrastructural resemblance between PT glands and CS (Milet et al., '80; Lopez et al., '84), the similarities in bioactivity form the main arguments in favour of the hypothesis that the CS of fish are homologous with the PT glands of the tetrapods. Not all available data support this hypothesis, however.

Several antisera raised against PTH do not cross-react with CS (Orimo et al., '84; our unpublished data), and differences in bioactivity between PTH and CS extracts have been reported, in particular for the effects on plasma calcium in rats, birds, and anurans. However, such differences certainly do not exclude phylogenetic homology. In our opinion, the main argument against homology is the probable difference in embryological origin of both glands. The PT glands are derived from the pharyngeal epithelium, which supplies the endocrine cells, and from the neural crest, which contributes to the connective tissue elements (LeLièvre and LeDouarin, '75). The CS probably originate from the epithelium of the nphric tubules (DeSmet, '62; Krishnamurthy, '67). This may imply that the similarities between PTH and CS substances represent a remarkable type of analogy in structure and bioactivity rather than true homology: the two different endocrine glands may produce hormones of the same family. In any case, the further study of these hormones will certainly contribute to the understanding of the evolution of hormones in general.

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LITERATURE CITED


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