Rapid Communication

Paradoxical Effect of 1.25 Dihydroxycholecalciferol on Osteoblastic and Osteoclastic Activity in the Skeleton of the Eel Anguilla anguilla L

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SUMMARY

Female mature eels (300 to 500 g) received one intraperitoneal injection of 1.25(OH)2D3 (10 μg). Their vertebral bone was compared, 8 h and 24 h after the injection, with vertebral bone of control mature female eels receiving solvent alone (ethanol). Sexual maturation in female eels induces a bone decalcification with hypercalcaemia and hyperphosphataemia. The control eels showed marked osteoclastic resorption and osteocytic osteolysis and the degree of mineralization of the intercellular substance decreased. Injection of 1.25(OH)2D3 into these female mature eels provoked as early as 8 h: 1) an increase in hypercalcaemia and hyperphosphataemia; 2) a major conversion of lining cells to osteoblasts and a stimulation of osteoblastic activity with new bone formation; 3) diminished osteoclastic resorption without changing osteocytic osteolysis or bone matrix mineralization.

Key words: 1.25 (OH)2D3 — Fish — Bone — Osteoblasts

INTRODUCTION

Parathyroids have not been described in fish (1) but two glands are principally involved in their phosphocalcic metabolism: the ultimobranchial body secreting calcitonin; and the Stannius corpuscles, hypocalcaemic glands situated on the kidney (2), which act with calcitonin at the site of the gills (3,4) and bone (5,6) to maintain calcium homeostasis. However, we generally forget that fish liver is extremely rich in vitamin D3 and very little is known of the function of the vitamin in these animals. Recent studies provide evidence that D3 is metabolized in fish. Thus, 25(OH)D3 is present in eel serum (7), bony fish have a specific plasma transport protein (α globulin) for 25(OH)D3 and 24R,25(OH)2D3 (8,9), and many species contain the 1-hydroxylase enzyme in their kidney (10). Further, 1.25(OH)2D3-like activity has been detected in eel tissues by competitive protein binding assay (10a). We have shown that exogenous 1.25(OH)2D3 increases plasma inorganic phosphate (11) and stimulates bone resorption in immature yellow and silver eels (12, 13). We now report studies of the effect of 1.25(OH)2D3 on osteoblastic activity in mature female silver eels whose skeleton was already greatly demineralized (14).

MATERIAL AND METHODS

Female silver eels (9 animals) obtained from Peronne (Somme) were placed in aerated fresh water and allowed to adapt to laboratory conditions at 18-20°C for one week. All were immature silver eels ranging in weight from 300 to 500 g. (Note that eels had never been found naturally mature). One week before the beginning of the experiment the eels were progressively adapted to sea water. Then they received intraperitoneal injections of carp pituitary extract (CPE, Stoller Fisheries, Spirit Lake, Iowa, USA) 3 times a week (1 mg/100 g body weight per injection) until complete maturation (three months) according to a method already described (15). When CPE treatment was stopped, just before spawning, 3 mature eels (controls) received one intraperitoneal injection of solvent alone (ethanol), 6 mature eels were given one intraperitoneal injection of 10 μg 1.25(OH)2D3 at the same time. Three eels injected with 1.25(OH)2D3 were killed 8 h after the injection and three others 24 h after the same injection. At the end of the experiment blood was collected from the ventral aorta and gonadal and body weight was measured.

For each animal caudal vertebrae were taken at the level of anus; the samples were fixed in alcohol 70% and colored with basic fuschin 1 %, embedded in methyl-methacrylate and cut into sections which were then ground manually to a thickness of 10 μm for a morphometrical study. Resorption surfaces and bone formation surfaces were localized by the identification tests proposed by Jaworsky (16). The measurement of the bone surfaces crenated and covered by osteoclasts (mononucleated and plurinucleated) and of the bone surface extent of the cuboidal form of osteoblasts (lining a thin new bone layer, colored by basic fuschin) was performed by the integrating method. We were using a Zeiss II integrating eyepiece (17,18); according to the Zeiss counting...
1.25(OH)₂D₃ injected into hypercalcaemic mature female eels (6) provoked as early as 8 h an increase in calcium and phosphate concentrations (Table 1) which did not rise further subsequently (24 h).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight g.</th>
<th>Gonadal weight g.</th>
<th>Plasma Ca (mg/l)</th>
<th>Serum P0₄ (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>716</td>
<td>366</td>
<td>16.6</td>
<td>5.3</td>
</tr>
<tr>
<td>ethanol</td>
<td>554</td>
<td>319</td>
<td>10.5</td>
<td>5.5</td>
</tr>
<tr>
<td>1.25(OH)₂D₃ 8 h</td>
<td>580</td>
<td>315</td>
<td>20.5</td>
<td>19.2</td>
</tr>
<tr>
<td>1.25(OH)₂D₃ 24 h</td>
<td>723</td>
<td>348</td>
<td>18.0</td>
<td>14.0</td>
</tr>
</tbody>
</table>

The effect of 1.25(OH)₂D₃ was studied on the various bone parameters. 1.25(OH)₂D₃ did not change the marked osteocytic osteolysis and the diminished degree of mineralization observed in mature female eels but it greatly increased the surface covered by osteoblasts and consequently the surfaces affected by osteoclastic resorption were reduced (Table 2).

In histological bone sections of mature female eels (Fig. 1) we saw that the bone surfaces were almost entirely crenated and lined by osteoclasts. Here, mononucleated osteoclasts very often close together. 8 h after 1.25(OH)₂D₃ injection we observed an increase in the number and activity of osteoblasts (Table 2). Prominent cuboidal osteoblasts with rounded apices covered a major part of the bone surface very often previously affected by osteoclastic resorption (Figs. 2-3).

DISCUSSION

It should be emphasised that the cells present in the bone of higher vertebrates are also present in the eel skeleton (20,22) and three different modes of demineralization can be observed (23). Sexual maturation in female teleosts, such as the eel (experimental maturation), and female conger eel naturally mature, greatly enhanced the three types of decalcification and provoked a marked increase in serum calcium and phosphate levels (22). The 1.25(OH)₂D₃ injected into mature female eels induced a further increase in the hypercalcaemia and hyperphosphataemia.