Central Laboratory and Medical Department E,
Frederiksberg Hospital, Copenhagen

HYDROXYPROLINE, CALCIUM, AND
MAGNESIUM CONTENTS OF BONES IN RATS AFTER
LONG-TERM TREATMENT WITH CALCITONIN

By
O. Helmer Sørensen, Inge Hindberg and
O. Bank-Mikkelsen

ABSTRACT

Intact, parathyroidectomized, and thyroparathyroidectomized rats were
treated for 30 days with 3 daily injections of calcitonin. At the end of the
experiment the contents of hydroxyproline, calcium, and magnesium were
determined in the clavicles and the tibias. Compared to the control groups,
which received the vehicle for calcitonin, no conclusive bone changes
could be observed in the treated rats.

It has been shown in a number of studies on isolated bone, that calcitonin acts
by inhibiting bone resorption (Aliapoullos et al. 1966; Friedman & Raisz 1965;
Reynolds & Dingle 1968; Nisbet & Nordin 1968; Flanagan & Nichols 1969). A
few in vitro assays indicate that calcitonin probably also increases bone forma-
tion (Gaillard & Thesingh 1968; Baxter et al. 1968).

Most acute in vivo experiments in rats are consistent with the prevention of
bone resorption as the major, if not the sole action of calcitonin (Milhaud et al.
O’Riordan & Aurbach 1968).

Wase et al. (1966) in acute perfusion experiments in rats, however, found
that calcitonin enhances the uptake of radioactive calcium in bone, in contrast
to the findings of Chauser et al. (1965), but in agreement with the recently

Foster et al. (1966) have shown in long-term studies in parathyroidectomized
rats that calcitonin treatment increases metaphyseal bone. In later experiments
the same investigators (Matrajt et al. 1968) obtained similar results in intact rats.

In earlier investigations (Hindberg & Sørensen 1971) we examined the effect of long-term calcitonin treatment in intact, parathyroidectomized, and thyroparathyroidectomized rats. We could not demonstrate any effect on bone, as evaluated by quantitative radiology, $^{45}$Ca studies, and measurement of the calcium and magnesium contents of isolated bones. However, we were unable to exclude an effect of calcitonin on bone collagen. In the present study we have determined the effect of calcitonin on the hydroxyproline, calcium, and magnesium contents in intact, parathyroidectomized, and thyroparathyroidectomized rats. The animals this time were treated with larger doses of calcitonin given at shorter intervals and during a longer experimental period. The animals were compared with similar groups of control rats, which received the vehicle for calcitonin.

**MATERIALS AND METHODS**

**Animals and diets**

Male albino rats of the Wistar strain, 6 weeks of age at the start of the experiment, were used. The animals were maintained on tap water and a hemisynthetic diet (Altromin®, supplied by Altromin GmbH, Lage, Germany) containing 1.4% Ca and 0.9% P.

Parathyroidectomy (PTX) and thyroparathyroidectomy (TPTX) was performed surgically under light ether anaesthesia. Animals with serum calcium values higher than 3.5 meq/l on the fifth postoperative day were rejected. The thyroparathyroidectomized rats received 300 μg of sodium liothyronine per litre of drinking water. Sham operations (SHAM) were done by exposure of the thyroids without further dissection.

The calcitonin-treated rats as well as the controls were divided into an intact, a parathyroidectomized, and a thyroparathyroidectomized group, each consisting of 10 animals. A few animals were excluded from the study because of unsatisfactory weight increase.

**Calcitonin**

Fresh porcine thyroid tissue was ground and lyophilized. Calcitonin was prepared from this material according to the procedure of Tenenhouse et al. (1965). The trichloracetic acid precipitate was dissolved in 0.02 N hydrochloric acid, and the trichloroacetic acid was removed by ion exchange. Sodium chloride and bovine albumin were added up to concentrations of 0.9% and 0.1% respectively. The purified extract contained 640 MRC mU/ml, assayed according to the procedure of Kumar et al. (1965). The experimental group was given subcutaneous injections of 130 MRC mU every eight hour for 30 days, while the control groups were injected with a similar volume of the vehicle.

**Bones**

Shortly after death the left clavicles and the right tibias of the animals were removed and carefully dissected free of connective tissue. After defatting in acetone followed by ether, the bones were dried to constant weight at 60°C. They were then hydrolyzed in 6 N HCl. An aliquot of the hydrolysates was ashed. The residues were dis-
solved and the calcium and magnesium concentrations were measured by atomic absorption spectrophotometry (I. L. Atomic Absorption Spectrophotometer 153). Another aliquot of the hydrolysates was used for hydroxyproline determinations by a slight modification of the method of Kivirikko et al. (1967).

The results were analyzed for statistical significance using Student's t test.

RESULTS

The mean weights ± sd for all rats increased from 127 ± 7 g to 232 ± 11 g during the 30 day-period without any significant differences between the 6 groups.

The dry bone weights of the left clavicles and the right tibias are shown in Tables 1 and 2. Except for a higher average weight of the tibias in the treated TPTX group, no significant differences between the calcitonin-treated rats and controls occurred.

Calcium, magnesium, and hydroxyproline concentrations per mg of bone are given in Tables 1 and 2. Minor and inconsistent differences between the treated animals and their controls were noted. With regard to the amounts of hydroxyproline, the contents were lower in the tibias of the TPTX animals than in the corresponding controls, but no other changes were observed. Concerning the mineral contents no regular pattern in the few alterations could be registered. The calcium and magnesium contents of the clavicles of the calcitonin-treated

Table 1.

Clavicles. Ca, Mg, and hydroxyproline per mg bone (mean ± sd).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Total bone weight mg</th>
<th>mg Ca per mg</th>
<th>μg Mg per mg</th>
<th>μg hydroxyproline per mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>Calcitonin</td>
<td>16.3 ± 1.7</td>
<td>0.210 ± 0.006</td>
<td>4.38 ± 0.27</td>
<td>34.8 ± 3.9</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>16.3 ± 1.7</td>
<td>0.222 ± 0.017</td>
<td>3.93 ± 0.56</td>
<td>33.0 ± 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>PTX</td>
<td>Calcitonin</td>
<td>14.9 ± 1.8</td>
<td>0.217 ± 0.012</td>
<td>5.06 ± 0.61</td>
<td>34.8 ± 4.2</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>15.1 ± 1.3</td>
<td>0.211 ± 0.024</td>
<td>5.28 ± 0.50</td>
<td>33.3 ± 4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>TPTX</td>
<td>Calcitonin</td>
<td>13.9 ± 0.8</td>
<td>0.210 ± 0.004</td>
<td>5.30 ± 0.25</td>
<td>37.7 ± 2.9</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>14.0 ± 1.4</td>
<td>0.199 ± 0.009</td>
<td>4.97 ± 0.26</td>
<td>34.9 ± 5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n.s.</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.02</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

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Table 2.
Tibias. Ca, Mg, and hydroxyproline per mg bone (mean ± sd).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Total bone weight mg</th>
<th>mg Ca per mg</th>
<th>μg Mg per mg</th>
<th>μg hydroxyproline per mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>Calcitonin</td>
<td>33.6 ± 1.4</td>
<td>0.211 ± 0.004</td>
<td>4.00 ± 0.09</td>
<td>28.2 ± 2.5</td>
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<tr>
<td></td>
<td>Control</td>
<td>34.1 ± 2.8</td>
<td>0.207 ± 0.012</td>
<td>4.24 ± 0.18</td>
<td>28.5 ± 2.1</td>
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<tr>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
<td>P &lt; 0.001</td>
<td>n.s.</td>
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<tr>
<td>PTX</td>
<td>Calcitonin</td>
<td>31.5 ± 2.6</td>
<td>0.211 ± 0.007</td>
<td>4.61 ± 0.42</td>
<td>31.7 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30.0 ± 2.2</td>
<td>0.221 ± 0.021</td>
<td>5.00 ± 0.33</td>
<td>30.2 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
<td>P &lt; 0.02</td>
<td>n.s.</td>
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<tr>
<td>TPTX</td>
<td>Calcitonin</td>
<td>30.8 ± 0.7</td>
<td>0.210 ± 0.007</td>
<td>4.78 ± 0.12</td>
<td>27.9 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>28.6 ± 1.7</td>
<td>0.205 ± 0.006</td>
<td>5.01 ± 0.34</td>
<td>30.9 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.01</td>
<td>n.s.</td>
<td>n.s.</td>
<td>P &lt; 0.002</td>
<td></td>
</tr>
</tbody>
</table>

TPTX rats were higher than those of their controls, while the opposite was the case for the magnesium concentration in the tibias of the SHAM and PTX rats.

**DISCUSSION**

In a previous study (Hindberg & Sorensen 1971) we were unable to demonstrate any effect of calcitonin on bone mineral in intact, parathyroidectomized, and thyroparathyroidectomized rats. The results are confirmed in the present study, where even larger doses of calcitonin were given at shorter intervals and for longer periods of time. Nor could we with the method used detect any influence of calcitonin on the hydroxyproline contents of bone.

So far the results of long-term treatment of experimental animals with calcitonin have been very conflicting. The first report on long-term treatment of rats with calcitonin came from Foster *et al.* (1966). They found that the hormone increased metaphyseal bone in parathyroidectomized animals, as evaluated by quantitative radiology and microscopy. In later descriptions of their experiments (Doyle *et al.* 1968) it appeared that in intact rats, they found a tendency to decreased bone mineralisation after calcitonin treatment. However, new microscopic examinations by the same group (Matrajt *et al.* 1968) showed that calcitonin significantly increased metaphyseal bone, in intact rats as well as in parathyroidectomized rats. In accordance with this, Tachdjian & Khan
(1967) observed increased amounts of calcium and collagen in intact and parathyroidectomized rats after two weeks of treatment but surprisingly enough they were unable to demonstrate similar changes in thyroparathyroidectomized rats (adequately supplied with thyroid hormone). In contrast Baylink et al. (1969) found in thyroparathyroidectomized rats that calcitonin inhibited both bone resorption and bone formation, the inhibition of bone resorption, however, being far greater (68%) than that of bone formation (24%).

Ewald & Tachjian (1967) observed a positive effect of calcitonin on fracture healing, but no changes occurred in the calcium concentrations of intact bones in calcitonin-treated rats.

Kalu et al. (1970) treated intact rats with 100 MRC mU of calcitonin daily for 20 days. Compared with buffer-treated control animals, the bones of calcitonin-treated animals did not contain significantly more collagen. When bone collagen resorption was increased by concomitant parathyroid hormone treatment, or by placing the animals on a low calcium diet, chronic calcitonin administration increased the total bone collagen. The animals in our experiment had a normal (SHAM) or reduced (PTX, TPTX) bone resorption so that our findings in this respect are in agreement with those mentioned above.

One must conclude that so far long-term administration of calcitonin has given varying results in animal experiments. One important factor in the response to calcitonin is age, young animals being far more sensitive to the hormone than old ones (Copp & Kuczerpa 1968; Sorensen et al. 1970). The animals in the present study were still in a stage of rapid growth, but they were nevertheless much older than the rats in which Foster et al. (1966) and Matrajt et al. (1968) found that calcitonin increases metaphyseal bone.

REFERENCES


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