THE EFFECT OF DIET AND AGE ON THE RESPONSE TO CALCITONIN IN RATS

By
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ABSTRACT

The hypocalcaemic effect of calcitonin has been found to be most pronounced in rats younger than approximately 10 weeks. If the rats are kept on a fixed diet for 24 h before an experiment, a low calcium/high phosphate diet is most suitable. When, however, the diet is maintained for longer periods, the growth rate must be taken into consideration as a high growth rate is essential for an optimal response to calcitonin.

The enhancing effect of an intravenous dose of phosphate on the response to calcitonin has been confirmed.

Calcitonin is a potent hypocalcaemic and hypophosphataemic hormone, which acts primarily by inhibiting bone resorption.

Harrison & Fraser (1960) found an increased bone resorption in rats kept on a calcium deficient diet, and Copp & Kuczerpa (1968) showed that a low calcium diet with adequate amounts of phosphate gives the best response to calcitonin. Hirsch (1968) pointed out that a phosphate rich diet enhanced the hypocalcaemic effect of calcitonin. In order to ascertain whether this was due to some action of dietary phosphate in the intestine, he injected small amounts of phosphate simultaneously with calcitonin which increased the response significantly.

In a preliminary study with diets of varying calcium and phosphate contents our findings were in fair agreement with those of Copp & Kuczerpa (1968), but it was observed that rats kept on a low calcium diet for 4 days showed, apart from a very good calcitonin response, a better growth rate than rats on a medium or high calcium diet. We therefore investigated the importance of growth rate in the calcitonin response and of dietary calcium for...
growth rate. In this connection we examined the effect of an iv dose of phos-
phate given at the same time as calcitonin.

Furthermore we tried to ascertain the optimal age of rats in a given cal-
citonin experiment, since it is not convenient to use very small animals when
large blood samples are required, although young rats have been found to be
more sensitive (Copp & Kuczerpa 1968; Phillippo & Hinde 1968; Sturridge &
Kumar 1968), as would be expected from their more rapid bone remodelling.

MATERIALS AND METHODS

Male Wistar rats were used throughout the investigation. Porcine calcitonin was
prepared according to the procedure of Tenenhouse et al. (1965) up to the formation
of the trichloroacetic acid precipitate. This was then dissolved in 0.02 N hydrochloric
acid containing 0.1% human albumin (electrophoretically pure, Behringwerke, A.G.).
The potency of the preparations was found to be 10 MRC (Medical Research Council)
milliunits per ml in a four-point assay with 6 rats in each group as described by
Kumar et al. (1965). MRC standard A calcitonin 65/101 was used as reference stan-
dard (kindly supplied by Dr. J. A. Parsons, Division of Biological Standards of the
National Institute for Medical Research, Mill Hill, London). The protein content of
the solution was 65 mg/100 ml as determined by the method of Lowry et al. (1951).
In all the experiments the rats received 10 MRC mU/100 g body weight sc. Blood
samples were taken by heart puncture under light ether anaesthesia both before and
60 min after the calcitonin injection. Serum calcium was determined by the method of
Wilkinson (1957). Unless otherwise indicated, the rats were kept on distilled water
and a standard diet (Altromin supplied by Altromin GmbH, Lage (Lippe), Germany)
with well defined contents of vitamins and minerals (1.4% Ca and 0.9% P) for at
least 4 days preceding the calcitonin injection. The response to calcitonin was tested
under the four sets of experimental conditions outlined below.

Experiment 1

Eight groups of 8 rats, each weighing 160 g, were kept on different hemisynthetic
diets for 5 days and were then given calcitonin. The calcium and phosphate contents
of the diets are shown in Fig. 1.

Experiment 2

Four groups of 18 rats (49 days of age, weights as shown in Tables 1 and 2) were
given an Altromin diet especially low in Ca and P, but otherwise identical with the
standard diet. The Ca and P contents indicated in Tables 1 and 2 were obtained by
adding K,H,PO, and calcium-gluconate. Nine rats from each group received the
calcitonin after 24 h (Table 1), the remaining 9 rats after 96 h (Table 2).

Experiment 3

Nine groups of 8 rats were used with ages varying from 38 to 210 days and with
accordingly varying weights from 80 to 500 g per animal. Furthermore, four groups
of 8 rats, 21 days of age, body weight 40 g, were used for the examination of the
response to calcitonin 30 and 60 min after iv or sc injection of the hormone. Because
of the small amounts of blood available from these rats, control values were obtained from groups receiving 0.02 N hydrochloric acid containing 0.1% albumin, instead of calcitonin.

Experiment 4

Three groups of 8 rats, 63 days of age, body weight 170 g, were used. The first group was given 60 μmoles of phosphate iv (0.2 ml 0.3 M NaH₂PO₄), the second group 60 μmoles of phosphate iv + calcitonin sc, and the third group 0.2 ml 0.9% saline iv + calcitonin sc.

RESULTS

As illustrated in Fig. 1 (Experiment 1) there is a good correlation between the average growth rate and the response to calcitonin. On the other hand, it is evident that the most significant hypocalcaemia was produced in the groups on low calcium diets (0.05–1.5% Ca), whereas the phosphate contents seemed to be of minor importance.

To investigate further the influence of the growth rate and of the dietary calcium/phosphate ratio, experiment 2 was performed. Table 1 shows that after 24 h on the various diets the effect of calcitonin was most pronounced in the group on the low Ca/high P diet, the difference from the groups on high

![Graph](/images/graph.png)

Fig. 1.

The response to calcitonin (mean ± sd) in relation to body weight increase of 160 g-rats after five days on the diet (8 rats per group). At each arrow percentage of Ca and P in the diet are given. Correlation coefficient 0.85, P < 0.001.
The effect of calcitonin in rats kept for 24 h on diets with different Ca and P contents (9 rats per group).

<table>
<thead>
<tr>
<th>% Ca</th>
<th>Diet</th>
<th>% P</th>
<th>Ca/P</th>
<th>Body weight (g ± sd) Before</th>
<th>After</th>
<th>-Δ Ca 1 h after calcitonin (mEq./l ± sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.19</td>
<td>0.29</td>
<td>0.66</td>
<td></td>
<td>142 ± 7</td>
<td>144 ± 10</td>
<td>0.86 ± 0.15</td>
</tr>
<tr>
<td>0.89</td>
<td>0.29</td>
<td>3.07</td>
<td></td>
<td>142 ± 10</td>
<td>143 ± 9</td>
<td>0.76 ± 0.23</td>
</tr>
<tr>
<td>0.19</td>
<td>0.91</td>
<td>0.21</td>
<td></td>
<td>143 ± 7</td>
<td>143 ± 10</td>
<td>1.02 ± 0.22</td>
</tr>
<tr>
<td>0.89</td>
<td>0.91</td>
<td>0.98</td>
<td></td>
<td>143 ± 10</td>
<td>143 ± 12</td>
<td>0.86 ± 0.31</td>
</tr>
</tbody>
</table>

Ca/low P and low Ca/low P being statistically significant (0.025 > P > 0.01 and 0.05 > P > 0.025 respectively). After 96 h on the diets, however, the rats given the low Ca/low P diet showed the best growth rate and also the most pronounced response to calcitonin, significantly better than the rats on the low Ca/high P diet (0.005 > P > 0.001) (Table 2). There is a striking contrast between the small weight increases of 0–2 g in the first 24 h as compared to the weight increases of up to 23 g in the 96 h experiment. When rats are placed on a new diet, they are often unwilling to eat until they have got accustomed to it. This is presumably the explanation for the small weight increases in all groups of rats during the first 24 h.

Fig. 2 (Experiment 3) shows that the sensitivity to calcitonin decreases with increasing age. However, up to an age of about 60 to 70 days there were no significant differences in sensitivity among the groups. Table 3 shows that the response to calcitonin in 21-day-old rats is of the same magnitude 30 and 60 min after the injection and that it is increased when the dose is administered iv instead of sc (P < 0.001).

Table 4 (Experiment 4) shows that the injection of a small dose of phosphate
The response to calcitonin in relation to age (mean ± sd; 8 rats per group).

Table 3.
The response to calcitonin in 21-day-old rats (8 rats per group).

<table>
<thead>
<tr>
<th></th>
<th>Serum Ca (mEq./l ± sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sc inj.</td>
</tr>
<tr>
<td>Controls</td>
<td>5.26 ± 0.07</td>
</tr>
<tr>
<td>(\frac{3}{4}) h after calcitonin</td>
<td>4.36 ± 0.15</td>
</tr>
<tr>
<td>1 h after calcitonin</td>
<td>4.31 ± 0.14</td>
</tr>
</tbody>
</table>

Table 4.
The effect of iv injection of phosphate on the response to calcitonin in 56-day-old rats (8 rats per group).

<table>
<thead>
<tr>
<th>Injection of</th>
<th>(-\Delta \text{Ca} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h after injection</td>
</tr>
<tr>
<td></td>
<td>(mEq./l ± sd)</td>
</tr>
<tr>
<td>Phosphate (60 (\mu)moles)</td>
<td>0.06 ± 0.12</td>
</tr>
<tr>
<td>Calcitonin + phosphate (60 (\mu)moles)</td>
<td>1.39 ± 0.29</td>
</tr>
<tr>
<td>Calcitonin + saline</td>
<td>0.93 ± 0.10</td>
</tr>
</tbody>
</table>
simultaneously with calcitonin increases the response significantly \((P < 0.001)\). Phosphate alone, in the dose used did not produce any hypocalcaemia.

**DISCUSSION**

Stenbock & Herting (1955) have shown that the growth rate in rats is very dependent upon the dietary calcium and phosphate contents, the most pronounced weight increase being observed on a low calcium diet with adequate amounts of phosphate. As bone remodelling is closely related to age and growth rate (Milhaud et al. 1963, 1967) it is not surprising that calcitonin induces the most significant hypocalcaemia in fast growing rats on a low calcium diet. Besides influencing the increase in body weight, a low calcium diet will bring about a secondary hyperparathyroidism with increased bone resorption (Harrison & Fraser 1960) resulting in an increased sensitivity to calcitonin. Cooper et al. (1967) found that rats fed a special low calcium diet for only one day showed a more pronounced response to calcitonin than rats fed the diet for four days. This might depend on a decreasing growth rate similar to that observed in this study in experiment 2 with the rats on the low calcium/high phosphate diet. After one day on the diet this group was most sensitive to calcitonin but after four days it showed a smaller response to calcitonin that the low calcium/low phosphate group, which had increased most in body weight.

Copp & Kuczerpa (1968) found a constantly decreasing sensitivity to calcitonin with increasing age. The differences in the degree of hypocalcaemia were, however, only small \(1\) h after the hormone injection whereas great differences were seen after \(3\) h. Phillippo & Hinde (1968) noted decreasing sensitivity to calcitonin in rats from \(4\) to \(12\) weeks of age. Sturridge & Kumar (1969) have shown that three-week-old rats are much more sensitive to calcitonin than six-week-old rats and that the peak response is obtained \(30\) min after iv administration. Milhaud et al. (1967), however, were unable to find any differences in bone catabolism and sensitivity to calcitonin in rats \(71-113\) days of age. Unfortunately they did not study rats younger than \(71\) days. In this study no differences in sensitivity to calcitonin were noted in rats up to ten weeks of age. The same degree of hypocalcaemia was seen \(30\) and \(60\) min after iv calcitonin injection in the three-week-old rats.

It has long been known that oral or parenteral administration of inorganic phosphate can lower the serum calcium concentration, and this has been used clinically in the treatment of severe hypercalcaemia (Albright et al. 1932; Goldsmith & Ingbar 1966; Herbert et al. 1966). The mechanism by which it reverses hypercalcaemia is not clear at present. In infusion experiments in rats, Pechet et al. (1967) demonstrated that phosphate does not inhibit the actions
of parathyroid hormone on bone, but rather stimulates bone formation or at least bone mineralization. Several investigations support the theory that the administration of inorganic phosphate reduces the serum calcium concentrations in patients with hypercalcaemia by inducing soft tissue calcifications (Albright et al. 1932; Herbert et al. 1966). However, it is difficult to explain how a dose of inorganic phosphate, which in itself does not affect the serum calcium concentrations, is able to increase the hypocalcaemic effect of calcitonin so impressively as found in this study.

It is concluded that when greater amounts of blood are required for serial analyses, seven- to ten-week-old rats are preferable. When only small amounts of calcitonin are available as for the determination of calcitonin in human blood (Gudmundson et al. 1969), very young rats should be used and given the hormone iv. In short term assays a low calcium/high phosphate diet is preferable, otherwise the rats should be put on the diet which gives the best growth rate.

REFERENCES


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