INFLUENCE OF GONADAL HORMONES ON THE ACTION OF CALCITONIN IN THE RAT

By

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ABSTRACT

The influence of short-term and long-term treatment with gonadal hormones on the response to calcitonin was investigated in the rat. Oestrogen-treatment, short-term as well as long-term, resulted in a reduced responsiveness to calcitonin. Long-term treatment with androgens enhanced the hypoccalcaemic effect of calcitonin in castrated rats of either sex, but reduced the effect in intact animals. No sex differences could be registered in the sensitivity to calcitonin, when intact animals were compared according to age, while marked differences were observed, when the animals were compared according to weight. There was a linear decrease in the response to calcitonin with increasing age in rats of both sexes. An intraperitoneal calcium load was followed by an acute rise in the serum calcium levels. The adult animals counteracted the hypercalcaemia more slowly than the young ones. Significant differences also occurred between male and female rats, the rise in the serum calcium concentration being much more pronounced in the latter group. The hypoccalcaemic activity of thyroid tissue from rats of both sexes and of various ages showed considerable variations, but no differences correlated to age or sex.

Bone metabolism is affected by sex hormones, but there are still different opinions about their precise effects. In the young growing rat oestrogens inhibit bone resorption (Day & Follis 1941; Budy et al. 1952), whereas testosterone apparently is of minor importance for bone resorption and formation in the rat (Budy et al. 1952; Tapp 1966). Schlüeter & Caldwell (1967) found female rats less responsive to calcitonin
than male rats. *Phillippo & Hinde* (1968) noted a general tendency for the male rat to respond to calcitonin better than females at any given age, but the differences in the response were not significant in all their experiments. The same investigators demonstrated, however, a synergistic action between oestradiol and calcitonin in intact and castrated female rats.

In recently published experiments *Ogata et al.* (1970) found that testosterone treatment greatly enhanced the hypocalcaemic response to calcitonin in male rats while the effect was less pronounced in female rats.

The purpose of the present investigation was to examine sex differences in sensitivity to calcitonin. The effect of short-term and long-term treatment with oestrogens or androgens was investigated both in intact and castrated rats. Recovery from hypercalcaemia was determined in male and female rats of various ages. Finally the thyroid content of calcitonin was determined in young and old rats of both sexes.

**MATERIALS AND METHODS**

Wistar rats of both sexes were used in all experiments. The animals were given a hemisynthetic diet containing 1.4% Ca and 0.9% P, Altromin®. They were kept fasting for 16 hours before the injections of calcitonin or calcium.

Porcine calcitonin was purified according to the procedure of *Tenenhouse et al.* (1965). The trichloroacetic acid precipitate was dissolved in 0.02 N HCl. After ion exchange on a column of Amberlite IRA 400, NaCl and bovine albumin were added to concentrations of 0.9% and 0.1%, respectively. The preparation was compared with a MRC standard B according to the procedure of *Kumar et al.* (1965). The estimated potency was 640 MRC mU/ml. The hormone was given subcutaneously in all experiments. Serum calcium was measured immediately before and one hour after the injection. Blood samples were taken by heart puncture under light ether anaesthesia. Analysis of serum calcium was carried out by the EDTA titration method (*Wilkinson 1957*). Bilateral gonadectomy was performed 8 days before the assay.

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

**Experiment 1**

Two groups consisting of 30 intact female rats and 30 intact male rats, all weighing 150 ± 10 g, were each divided into 3 subgroups of 10 rats. The first group of either sex received 500 μg of testosterone propionate twice daily for 2 days before the experiment, the second group was injected with 10 μg of oestradiol benzoate at similar intervals, while the third group only had injections of sesame oil. The hypocalcaemic effect of 20 MRC mU of calcitonin was compared in the 6 groups.

**Experiment 2**

Sixty male rats, 5 weeks of age, were divided into 2 main groups: 30 intact and 30 castrated rats. Each group was further subdivided into 3 groups, which received the following injections on the first and on the tenth day of the experiment: Group 1:
vehicle (sesame oil), group 2: 25 mg of testosterone oenanthate (Testoviron-Depot®, Schering), and group 3: 10 mg of oestradiol undecylate (Progynon-Depot®, Schering). Sixty female rats of the same weights were divided and treated according to a similar scheme. On the 21st day all the rats received a single injection of calcitonin (20 MRC mU/100 g body weight) and the hypocalcaemic responses were measured.

Experiment 3
The effect of calcitonin was studied in 7 groups of rats, each group consisting of 8 males and 8 females. The ages of the groups ranged from 21 to 140 days. The animals were given a single dose of calcitonin (20 MRC mU/100 g body weight) and the hypocalcaemic responses in male and female rats were compared.

Experiment 4
The effect of an acute calcium load was studied in groups of 10 rats of either sex at various ages between 3 and 18 weeks. The animals were injected intraperitoneally with isotonic CaCl₂, 5 mg Ca/150 g body weight. Serum calcium was determined immediately before and 60 min after the injection. Two groups of 35 male rats, 4 and 11 weeks of age, respectively, were given a similar calcium load as described above. The serum calcium concentration was determined in groups of 5 rats, before and at regular intervals afterwards up to 120 min after the injection.

Experiment 5
The calcitonin contents were measured of thyroid glands from female and male rats at various ages. The thyroid glands from groups of 5 rats were removed under ether anaesthesia, pooled, weighed and homogenized in cold 0.1 x HCl (1 ml HCl/mg thyroid tissue). Two to 4 dilutions of the thyroid homogenate were assayed in 6 male rats (weight 100 g) at each dose level.

RESULTS
The serum calcium levels were not influenced by castration or treatment with gonadal hormones (Tables 1 and 2). As can be seen from Table 1 all three groups of male rats responded to calcitonin better than the corresponding female rats (oil-treated: \( P < 0.005 \), androgen-treated: \( P < 0.01 \), oestrogen-treated: \( P < 0.001 \)). The short-time experiments with sex hormones showed a reduced sensitivity in oestrogen-treated rats as compared with the control animals (males: \( P < 0.05 \), females: \( P < 0.01 \)) while androgen treatment did not affect the response to calcitonin.

Treatment of intact and castrated rats for a longer period with gonadal hormones changed their sensitivity to calcitonin considerably (Table 2). Oestrogens thus, in all rats, significantly reduced the response (\( P < 0.001 \) in both groups of male rats and in intact female rats, \( P < 0.005 \) in castrated female rats). Androgen treatment enhanced the hypocalcaemic effect in
Table 1.
Short-term treatment with sex hormones. Starting level and fall in serum Ca (mEq./l) 1 h after sc injection of 20 MRC mU of calcitonin into 150 g rats (mean ± sd; 10 rats per group).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Male rats</th>
<th></th>
<th>Female rats</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before calcitonin</td>
<td>−Δ Ca</td>
<td>Before calcitonin</td>
<td>−Δ Ca</td>
</tr>
<tr>
<td>Oil</td>
<td>5.27 ± 0.13</td>
<td>0.90 ± 0.23</td>
<td>5.26 ± 0.12</td>
<td>0.59 ± 0.13</td>
</tr>
<tr>
<td>Androgen</td>
<td>5.24 ± 0.06</td>
<td>0.87 ± 0.18</td>
<td>5.28 ± 0.06</td>
<td>0.63 ± 0.18</td>
</tr>
<tr>
<td>Oestrogen</td>
<td>5.37 ± 0.11</td>
<td>0.70 ± 0.17</td>
<td>5.30 ± 0.09</td>
<td>0.42 ± 0.11</td>
</tr>
</tbody>
</table>

Castrated rats of both sexes ($P < 0.01$), but resulted in inhibition in intact animals in the doses used (males $P < 0.001$, females $P < 0.05$). As in the previous experiment intact control males were more responsive to calcitonin than intact females of the same weight ($P < 0.001$). Castrated control animals of both sexes showed a reduced sensitivity as compared with intact animals (males: $P < 0.001$, females: $P < 0.025$).

The increase in body weight were also changed by treatment with large doses of gonadal hormones (Table 3). Oestrogens significantly inhibited the growth rate in all groups of rats. Strangely enough the intact male rats treated with androgens in this experiment showed a retarded growth rate, as compared with the control rats. In other experiments, carried out in a similar way, we found no differences in the growth rate between these two groups of rats. Fig. 1 shows that there was a good correlation between body weight increase and the response to calcitonin ($r = 0.86$, $P < 0.001$).

No sex differences was observed in the hypocalcaemic effect of calcitonin, provided the rats were compared by age and not by weight, as shown in Fig. 2. The same degree of hypocalcaemia was registered in the 3 youngest groups of rats of both sex (aged 21 to 42 days), while in the older animals there was a linear decrease in sensitivity with increasing age.

An intraperitoneal calcium load was followed by an acute rise in the serum calcium. The load was dosed per g body weight and resulted in a higher degree of hypercalcemia in the old rats (11-week-old) than in the young animals (4-week-old), Fig. 3. In the young animals, the rate of return to normal and even subnormal levels was much faster than in the old ones.

The serum calcium values, measured one hour after an intraperitoneal calcium load to rats of both sexes and at different ages, are shown in Fig. 4.
Table 2.
Long-term treatment with sex hormones. Starting level and fall in serum Ca (mEq/l) 1 h after sc injection of 20 MRC of calcitonin per 100 g body weight (mean ± sd; 10 rats per group).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Male rats</th>
<th>Female rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Castrated</td>
</tr>
<tr>
<td></td>
<td>Before calcitonin</td>
<td>−Δ Ca</td>
</tr>
<tr>
<td>Oil</td>
<td>5.36 ± 0.07</td>
<td>1.10 ± 0.09</td>
</tr>
<tr>
<td>Androgen</td>
<td>5.23 ± 0.08</td>
<td>0.98 ± 0.09</td>
</tr>
<tr>
<td>Oestrogen</td>
<td>5.30 ± 0.08</td>
<td>0.80 ± 0.11</td>
</tr>
</tbody>
</table>
Table 3.

Body weight in g (mean ± sd; 10 rats per group) before and after long-term treatment with sex hormones.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Male rats</th>
<th></th>
<th>Female rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Castrated</td>
<td>Intact</td>
</tr>
<tr>
<td></td>
<td>At start</td>
<td>At end</td>
<td>At start</td>
</tr>
<tr>
<td>Oil</td>
<td>116 ± 6 → 217 ± 13</td>
<td>104 ± 8 → 156 ± 26</td>
<td>106 ± 8 → 158 ± 8</td>
</tr>
<tr>
<td>Androgen</td>
<td>110 ± 3 → 185 ± 15</td>
<td>106 ± 6 → 163 ± 9</td>
<td>107 ± 6 → 161 ± 8</td>
</tr>
<tr>
<td>Oestrogen</td>
<td>113 ± 5 → 132 ± 7</td>
<td>108 ± 7 → 111 ± 9</td>
<td>107 ± 7 → 126 ± 10</td>
</tr>
</tbody>
</table>
It is clear that the adult animals counteracted the calcium load more slowly than the young ones. No consistent sex differences could be registered in the young animals. In the adult groups, however, a significant difference occurred between males and females, the rise in the serum calcium concentration being far more pronounced in the female rats.

It is possible that the different responses to an acute hypercalcaemia in immature and adult rats and in males and females might depend on the varying amounts of calcitonin stored in the thyroid gland and available in an emergency situation. The thyroidal contents of hypocalcaemic activity were there-
Fig. 3.
Serum Ca following an ip Ca load (5 mg/150 g body weight) in male rats. Each point represents the mean ± SD of 5 rats.

fore measured in male rats weighing 50, 200, 300, and 400 g and in female rats weighing 50, 200, and 300 g. As illustrated in Fig. 5 the dose-response curves showed a good parallelism ($r = 0.89, P < 0.001$). Repeated assays with thyroid tissue from other rats of similar size showed considerable variations in the hypocalcaemic activities found, but no difference due to age or sex (Fig. 5).

**DISCUSSION**

Several investigations have shown that calcitonin primarily acts by inhibiting bone resorption (Aliapoulios et al. 1966; Friedman & Raisz 1965; Milhaud et al. 1965). The hormone consequently induces the most pronounced hypocalcaemia in conditions characterized by a high rate of bone catabolism such as hyperthyroidism (Bijvoet et al. 1968), hyperparathyroidism (Sørensen et al. 1970a) and rapid growth in young animals (Copp & Kuczerpa 1968; Sørensen et al. 1970b).

It has been found in a number of studies that treatment of growing rats with oestrogens depresses bone resorption (Day & Follis 1941; Budy et al. 1952; Lindquist et al. 1960). It is therefore not surprising to find a less pronounced hypocalcaemia following a calcitonin injection in oestrogen-treated rats in
which bone catabolism is reduced. In the present study this effect was observed in all rats both with regard to the short-term assay and the long-term experiments. The results are in contrast to the findings of Phillippo & Hinde (1968), who found an enhanced hypocalcaemic response to calcitonin in oestrogen-treated rats.

A number of experiments have shown a lack of effect of androgens on bone metabolism (Turner et al. 1941; Budy et al. 1952; Tapp 1966) but the subject has not been studied as intensively as in the case of the oestrogens. Ogata et al. (1970) recently found an enhanced effect of calcitonin in testosterone-treated rats most particularly in male rats. In the present study long-term treatment with androgens increased the hypocalcaemic response to calcitonin in castrated rats of both sexes. However the sensitivity was reduced in intact animals. Kochakian et al. (1950) in their studies on the anabolic effect of androgens, have found a similar reduced effect of testosterone propionate in intact rats as compared to castrated rats. Ogata et al. (1970) found a decreased response to calcitonin after gonadectomy in male rats and a slightly increased sensitivity

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Fig. 4.
Serum Ca 1 h after an ip Ca load (5 mg/150 g body weight) in rats of either sex (mean ± s.d.; 10 rats per group).
The hypocalcaemic activity of thyroid tissue from rats of either sex and at various ages. Each point represents the mean response of 6 male rats weighing 100 g. Correlation coefficient: \( r = 0.89 \) \((P < 0.001)\) for the groups to the left and \( r = 0.75 \) \((P < 0.001)\) for the groups to the right.

in castrated female rats. In this investigation the results showed a reduced effectiveness of calcitonin in castrated rats of both sexes. As shown by Hansard & Crowder (1957) and Milhaud et al. (1963, 1967) bone catabolism is closely correlated to growth. In an earlier investigation concerning diet and response to calcitonin we demonstrated that the most pronounced effect was seen in the most rapidly growing animals (Sorensen et al. 1970b). Similar results were obtained in the present study. Castration alone and oestrogen treatment of intact and castrated rats inhibited the growth rate. This was followed by a diminished sensitivity to calcitonin. In the doses used long-term treatment with androgens increased the growth rate and thereby the response to calcitonin in castrated rats of either sex, while the opposite was the case in intact male rats.

As demonstrated in several experiments (Copp & Kuczerpa 1968; Orimo 1967; Phillippo & Hinde 1968; Sorensen et al. 1970b) the effect of calcitonin decreases with increasing age. This phenomenon was also observed in the present study, except in the youngest groups of rats, among which no differences in the response were seen. Schluter & Caldwell (1967) showed that the female rat is less sensitive to calcitonin than the male rat. However, they compared the animals by weight and not by age. Phillippo & Hinde (1968) obtained varying results, when the two sexes were compared according to
weight. In the same experiments they were able to demonstrate sex differences in the response to calcitonin, when the rats were compared according to age. This was also the case in the experiments of Ogata et al. (1970). Cooper et al. (1967) found that rats of either sex were equally responsive. In the present study significant differences in sensitivity were seen when the hormone was given to rats of both sexes and of similar weights. This was not the case when animals of the same age were compared and when calcitonin was administered according to weight.

Talmage et al. (1965) demonstrated that the thyroid gland plays an important role in restricting hypercalcaemia induced in rats by the injection of calcium chloride. Their findings have been confirmed by other investigators (Gittes & Irvin 1966; Bronner et al. 1967; Sorensen 1970). Orimo (1967) found a delayed recovery from hypercalcaemia in old rats as compared to young animals, which suggested a decreased response of bone to calcitonin with increasing age. These results were confirmed in the present study. In addition we observed that the delay was greater in old female rats than in old male animals of the same age, which might depend on differences in the response of bone to calcitonin or differences in the secretion rate of the hormone. Other factors may also play a role, such as the rate of incorporation of calcium into bones and soft tissues. Hruza (1969) clearly showed that $^{45}$Ca is incorporated faster into most tissues in young rats than in old animals and in males more than in females. Another important factor in the reaction to an acute calcium load is the size of the miscible calcium pool. This is relatively greater in young animals than in adults (Bell 1967), a fact which might explain the lower peak in the serum calcium concentration in the young animals following a calcium load.

That calcitonin plays a considerable role in the recovery from hypercalcaemia can be seen from the fact that the serum calcium in the youngest rats, one hour after the calcium load, had reached values which were lower than the starting levels. This can only be explained by an increased secretion of calcitonin. In agreement with this it has been shown that hypercalcaemia reduces the thyroidal content of calcitonin (Gittes et al. 1968; Frankel & Yasumura 1970). Cooper (1968) found a relationship between the sensitivity to calcitonin and the concentration of the hormone in the thyroid gland, the less sensitive strains of rats having the largest amounts of intrathyroidal hormone. Frankel & Yasumura (1970) found significantly reduced concentrations of calcitonin in 5- and 15-day-old rats as compared to 60-day-old rats, whereas the concentrations in 30-, 120-, and 360-day-old animals did not differ from that of the 60-day-old rats. We did not examine the thyroidal contents of calcitonin in rats younger than 3 weeks, but from that age and up to the age of 20 weeks we were not able to detect any differences.
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