INTERRELATIONSHIP BETWEEN CALCITONIN AND PARATHYROID HORMONE IN INTACT, THYROIDECTOMIZED, PARATHYROIDECTOMIZED, AND THYROPARATHYROIDECTOMIZED RATS

By

O. Helmer Sørensen

ABSTRACT

Calcitonin is capable of completely suppressing the hypercalcaemic effect of parathyroid hormone but not irreversibly. The parathyroid hormone extract used was the commercial preparation Para-Thor-Mone® (Lilly). When injected simultaneously, the parathyroid hormone had no influence on the hypocalcaemia induced by calcitonin one hour after the injection. The parathyroid hormone produced a hypercalcaemia of up to 42 h duration in rats without thyroids. If the thyroid gland was present the serum calcium did not exceed 5.5 mEq/l, probably because of an increased secretion of calcitonin. A transitory hypercalcaemia followed by a hypocalcaemia was seen in intact rats after an intraperitoneal calcium load. The hypocalcaemia could be explained by the counteracting secretion of calcitonin.

Sanderson et al. (1960) showed that thyroparathyroidectomized dogs were not capable of restoring their original serum calcium level as rapidly as normal dogs after infusion of either calcium or EDTA (ethylene diamine tetraacetate). Failure to control hypocalcaemia was readily explained by the lack of parathyroid hormone, whereas the impaired control of hypercalcaemia was difficult to understand. The investigators considered lack of a thyroid factor as a possible explanation, but treatment with thyroid hormone did not change the results. Two years later Copp et al. (1962) were able to explain the findings of Sanderson et al. (1960) by showing that the thyroid-parathyroid complex in dogs contains a rapidly acting calcium-lowering hormone, which was called calcitonin. Calcitonin exerts its hypocalcaemic effect by inhibiting bone re-
Table of calcium and chloride) every units thyroid Calcitonin (1965). The response of serum calcium to parathyroid hormone is reduced by concomitant administration of calcitonin (Milhaud & Moukhtar 1966; Evanson et al. 1967; Anast et al. 1967; Morii & DeLuca 1967). However, calcitonin does not act by inhibiting parathyroid hormone as it is also effective in parathyroidectomized animals (Hirsch et al. 1963; Gudmundsson et al. 1966). The purpose of this investigation was to examine the interrelationship between the parathyroid hormone and calcitonin in intact, parathyroidectomized, thyroidectomized and thyroparathyroidectomized rats.

MATERIALS AND METHODS

Male Wistar rats, 6-7 weeks old and weighing 160-180 g, were used in all experiments. The animals were fed ad lib. a semisynthetic diet containing 0.9% phosphorus and 1.3% calcium (Altromin supplied by Altromin Gmtr, Lage, Germany). After thyro-parathyroidectomy or parathyroidectomy, drinking water was replaced by a 4% calcium lactate solution for two days. All the animals were fasted for the last eight hours before the experiments. Thyroparathyroidectomy (TPTX) or parathyroidectomy (PTX) was performed surgically under the anaesthesia. The serum calcium was determined on the third postoperative day, i.e. at the start of the experiment, and rats with values above 4 mEq./l were rejected. Thyroidectomy (TX) was done by performing a total thyroparathyroidectomy followed by immediate transplantation of the two parathyroid glands into the sternomastoid muscles. The parathyroids were carefully dissected free from thyroid tissue. Rats with serum calcium values lower than 4.5 mEq./l on the fifth day after the transplantation were discarded. Control rats were sham operated (SHAM) by exposure of the glands without further dissection. Calcitonin was extracted from porcine thyroid glands as described by Tenenhouse et al. (1965) to the stage of trichloroacetic acid precipitation followed by dissolution in 0.02 N HCL containing 0.1% bovine albumin. The activity was 30 MRC (Medical Research Council standard) mU per ml as determined by the method of Kumar et al. (1965). The hormone was injected sc in a dose of 15 MRC mU per rat. The parathyroid extract used was the commercial preparation Para-Thor-Mone (Lilly), containing 100 USP parathyroid units per ml. It was injected sc in doses of 150 USP units per rat. The TX and TPTX animals received 3 μg of sodium iothryonine sc every 24 h. Calcium loading was performed by injecting ip 5 mg calcium (as calcium chloride) per 100 g body weight.

Blood was taken by heart puncture under light ether anaesthesia. The serum calcium concentrations were determined by the method of Wilkinson (1957).

RESULTS

Table 1 illustrates the effect of simultaneous injection of calcitonin and parathyroid hormone into intact rats. The controls received 2 ml of isotonic saline sc. Parathyroid hormone did not change the hypocalcaemic response to sc dose of calcitonin 1 h after the injection of the hormones. Injection of parathyroid
Table 1.
Comparative studies of serum calcium in intact rats. Each value represents the average of 8 rats. PTH = parathyroid hormone, CT = calcitonin.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum calcium mEq./l ± sd 1 h after the injection</th>
<th>Difference from controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (0.9% saline)</td>
<td>5.49 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>PTH 150 U</td>
<td>5.36 ± 0.10</td>
<td>*P &lt; 0.005</td>
</tr>
<tr>
<td>PTH 150 U + CT 15 MRC mU</td>
<td>4.44 ± 0.10</td>
<td>*P &lt; 0.001</td>
</tr>
<tr>
<td>CT 15 MRC mU</td>
<td>4.38 ± 0.09</td>
<td>*P &lt; 0.001</td>
</tr>
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Fig. 1.
Response to a single injection of 150 USP U of parathyroid hormone at time zero followed by injection of 15 MRC mU of calcitonin 8 h later in TPTX rats. Each point represents the average value ± sd of 8 rats.

Hormone alone did not induce any hypercalcaemia in intact rats in 1 h, but on the contrary a slight but significant hypocalcaemia.

Fig. 1 shows how calcitonin is capable of changing the hypercalcaemic effect of parathyroid hormone in TPTX rats. The animals were given parathyroid hormone at the start of the experiment. Eight hours later they received an injection of calcitonin, which lowered their serum calcium significantly, but not irreversibly, since their calcium levels were elevated again after 18 h.

Fig. 2 illustrates the changes in the serum calcium concentration in SHAM, TPTX, TX and PTX rats 5 h after the injection of parathyroid hormone. No changes were registered in the SHAM group. The serum calcium rose in the PTX rats from low to normal values. The TPTX animals also had low starting levels, but their serum calcium concentration increased to hypercalcaemic
Fig. 2.
Response to a single injection of parathyroid hormone in SHAM, TX, PTX, and TPTX rats. Each bar represents the average of 8 rats. sd is indicated for each group by vertical lines. □ before the injection, □ 5 h after the injection.

values. This was even more pronounced in the TX group, which, however, had normal starting levels.

In a preliminary experiment SHAM and TPTX rats were given the usual dose of parathyroid hormone. Their serum calcium values before and 24 h after the injection are presented in Table 2.

No changes occurred in the SHAM group, whereas the calcium concentration was still significantly elevated in the TPTX animals 24 h after the injection.

In order to investigate further this prolonged effect of parathyroid hor-

Table 2.
Response to parathyroid hormone in SHAM and TPTX rats. Each value represents the average of 8 rats.

<table>
<thead>
<tr>
<th></th>
<th>Serum calcium mEq./l ± sd</th>
<th>difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before the inj.</td>
<td>24 h later</td>
</tr>
<tr>
<td>SHAM</td>
<td>5.17 ± 0.04</td>
<td>5.13 ± 0.10</td>
</tr>
<tr>
<td>TPTX</td>
<td>3.49 ± 0.24</td>
<td>6.31 ± 0.52</td>
</tr>
</tbody>
</table>
monoc in rats with and without their thyroids a similar dose was given to three groups of animals: TX, PTX and SHAM rats. The serum calcium was determined before, $2^{1/2}$, 5, 24 and 42 h after the injection. The results are shown in Fig. 3. The serum calcium level remained constant in the SHAM group, while in the TX rats it increased to levels much higher than normal with a decline after 24 h, and in the PTX animals the calcium concentration increased to normal values, although not higher, for more than 42 h. After $2^{1/2}$ h the parathyroid hormone had already produced a definite effect in the TX and PTX animals.

In the next experiment the hypercalcaemia was produced by an intraperitoneal calcium load. Three groups were examined: SHAM, PTX and TPTX rats. There was a very high mortality (50-70 per cent), 3 to 10 h after the calcium load, in the PTX and TPTX rats in contrast to the SHAM animals, which all survived. The serum calcium concentration was controlled immediately after death in several of the animals. It did not in any case exceed the values found $1/2$ hour after the injection. The results are shown in Fig. 4. The hypercalcaemia was of short duration in all the animals and of the same magnitude in contrast to the hypercalcaemia induced by parathyroid hormone. It is of interest that the five-hour values in the SHAM operated group are lower than the values before the injection.

In another experiment smaller doses of calcium were given (3 mg calcium per 100 g body weight) and the calcium concentrations in the blood only determined just before and one hour after the injection. The increases were as follows: SHAM rats: $0.68 \pm 0.22$ (from 4.93 to 5.61 mEq./l), PTX rats:

![Fig. 3](image_url)

Response to a single injection of parathyroid hormone in SHAM, TX, and PTX rats.
Each point represents the average $\pm$ sd of 9 animals.
**Fig. 4.**
Response to injection of 5 mg of Ca intraperitoneally in SHAM, PTX, and TPTX rats. Each bar represents the average of 8 rats. sd is indicated for each group by vertical lines.

0.99 ± 0.22 mEq./l (from 3.63 to 4.62 mEq./l), TPTX rats: 1.55 ± 0.31 mEq./l (from 3.13 to 4.68 mEq./l). Each value represents the average of eight rats. The differences between the increase in TPTX rats and the increases in the SHAM and PTX rats were now highly significant ($P < 0.001$, $P < 0.001$), while the difference between the SHAM and the PTX rats was less significant ($P < 0.01$).

**DISCUSSION**

The results in this study are in agreement with those of *Talmage et al.* (1965), who demonstrated that TX and TPTX rats after an intraperitoneal calcium load reached a significantly higher serum calcium level and recovered more slowly from the hypercalcaemia than intact and PTX rats. Their findings have been confirmed by others (*Bronner et al.* 1968; *Gittes & Irvin* 1966). Strangely enough *Talmage et al.* (1965) also found differences between TPTX PTX rats at serum calcium levels far below 5 mEq./l, i.e. in cases where the secretion of calcitonin should be completely interrupted and hence the presence or absence of the thyroid gland should be unimportant.

*Gittes & Irwin* (1965) found that the hypercalcaemic effect of parathyroid hormone 6 h after the injection was greatest in TPTX or TX rats, intermediate in PTX rats and smallest in intact animals. Their investigations were re-examined by *Hirsch & Munson* (1966), who showed that the increase in serum calcium 6 h after the administration of the hormone was essentially the same in PTX and SHAM rats and much smaller than in TPTX rats.
Anast et al. (1967) demonstrated a slower rise in the serum calcium concentration in PTX rats than in TPTX rats during a long term infusion of parathyroid hormone. All these findings point to the thyroid origin of calcitonin.

Melick et al. (1965) estimated the half-life of parathyroid hormone in the plasma of the rat as being about 20 min, but the effect of the hormone may last for up to 24 h. If the prolonged elevation of the serum calcium after injection of parathyroid hormone in PTX, TX, and TPTX rats in the present study does not depend on a continuous parathyroid hormonal effect, but on a slow regulation of the diffusion equilibrium between plasma and bone mineral, one would expect to find a hypercalcaemia of a similar duration when the same degree of hypercalcaemia has been induced by a calcium load. This was, however, not the case. The calcium values returned to preinjection levels in less than 5 h in SHAM, PTX, and TPTX rats.

Sherwood et al. (1968) have shown by radioimmunoassay that the parathyroid hormone secretion is inversely proportional to the concentration of calcium in the blood and ceases at concentrations above 6 mEq/l. The exact opposite is true of calcitonin, the secretion being directly proportional to the blood calcium (Care et al. 1968) and not measurable at concentrations below 6 mEq/l. This dual hormonal feedback system controls the very constant serum calcium level. Normal rats are notoriously insensitive to parathyroid hormone (Pugsley 1982). This is not the case in TX animals, in which the counteracting calcitonin secretion is eliminated by thyroidectomy. The results in this study showed a sustained hypercalcaemia in these animals up to 42 h after the parathyroid hormone injection (Fig. 3). An effect of similar duration was observed in PTX animals in which the serum calcium concentration was normalized, but never exceeded normal values, no doubt because of increased calcitonin secretion. As this secretion is interrupted at serum calcium concentrations lower than about 6.0 mEq/l, the parathyroid hormone can thus exert its effect in PTX rats for the same period of time as in TX animals.

Intact rats will counteract a calcium load by increased secretion of calcitonin. This is illustrated in Fig. 4, where the SHAM rats with average starting serum calcium values of 5.29 ± 0.08 mEq/l as a result of hypersecretion of calcitonin, reach a level as low as 4.38 ± 0.35 mEq/l 5 h after the intraperitoneal calcium load. The very low 24-h values in the PTX and TPTX rats in this experiment presumably depend upon the fact, that the animals would not eat the usual amounts of food.

Calcitonin is capable of suppressing the hypercalcaemic effect of parathyroid hormone. This is not due to an irreversible inactivation, which is clearly illustrated in Fig. 1 where the calcitonin induced hypocalcaemia is only transitory and is followed by a sustained parathyroid hormone effect.

The slight decrease in serum calcium found 1 h after injection of parathyroid hormone into intact rats (Table 1) might be due to small impurities of
calcitonin in the commercial parathyroid preparation, as reported previously by Copp et al. (1962).

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