PARATHYROID HORMONE SECRETION
IN DISORDERS OF CALCIUM METABOLISM
STUDIED BY MEANS OF EDTA

By

J. H. Lockefeer, W. H. L. Hackeng
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ABSTRACT

1. In 22 of 28 cases of primary hyperparathyroidism (PHP) the rise in the serum immunoreactive parathyroid hormone (IRPTH or PTH) level observed in response to lowering of the serum calcium by EDTA, exceeded that obtained in 8 control subjects. In 5 of these 22 patients who were studied again after parathyroidectomy the supranormal response was abolished. Fifteen of these 22 hyper-responsive PHP patients had basal IRPTH levels not exceeding the highest level in the controls and that of other groups of patients investigated (idiopathic hypercalciuria, non-parathyroid hypercalcaemia, operated PHP).

2. Fourteen of the 22 hyper-reactive patients with PHP did not show hypocalcaemia during the infusion of EDTA.

3. The extent of the release of PTH elicited by EDTA in cases of PHP does not as yet allow a prediction of the amount of pathological parathyroid tissue present, although all the PHP patients showing a normal release of PTH had a relatively small mass of parathyroid tissue (up to about 1 g) subsequently removed.

4. In 9 cases of nephrolithiasis (8 of whom had idiopathic hypercalciuria) and in 7 cases of non-parathyroid hypercalcaemia, a normal PTH release was found.

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For a considerable number of cases of hyperparathyroidism (20–70 %) normal basal levels of serum immunoreactive parathyroid hormone (IRPTH) have been reported by various groups (Berson & Yalow 1966; Lequin et al. 1970; Arnaud et al. 1971; O'Riordan et al. 1972). However, with their assay Arnaud et al. (1971) were able to distinguish patients with PHP and normal PTH levels from normal subjects by plotting the basal PTH against the calcium concentrations, as in their experience PTH secretion is suppressed by non-parathyroid hypercalcaemia. Calcium infusion studies have shown that in primary hyperparathyroidism the parathyroid glands generally do not show an absolute functional autonomy (Pronove & Bartter 1961; Potts et al. 1971; Murray et al. 1972). However, the extent of the inhibition of the release of parathyroid hormone (PTH) appears to be subnormal in hyperparathyroidism in as much that the PTH concentrations did not become undetectable in the study of Potts et al. (1971). In view of the frequency of the finding of a normal basal serum PTH level in PHP and on the basis of a) the relative lack of autonomy of the PTH secretion found in PHP and b) the excessive release of PTH from the parathyroid glands in the secondary parathyroid hyperplasia of parturient cows, reported to occur on spontaneous development of hypocalcaemia (Potts et al. 1968) we studied the response of serum IRPTH to infusion of EDTA in controls, in patients with surgically proven PHP before and after operation, in patients with nephrolithiasis with or without hypercalciuria and in patients with hypercalcaemia caused by non-parathyroid disease. While the present study was in progress Potts and coworkers reported in PHP a 1.5–7 fold increase of serum IRPTH induced by the infusion of EDTA (Potts et al. 1971; Murray et al. 1972), while Wen Chen et al. (1972) compared the IRPTH responses of control subjects and hyperparathyroid patients to EDTA.

MATERIALS AND METHODS

After an overnight fast the trisodium salt of EDTA was administered in a dose of 50 mg per kg body weight in 500 ml 5 % dextrose with the addition of 30 ml 2 % novocaine (Parfitt 1969) given by intravenous infusion over 2 h starting at 9 a.m. No dietary measures were taken. Blood samples were taken at zero time, at 30 min intervals up to 2–4 h, at 2 intervals up to 12 h and after 24 h. PTH was measured by radio-immunoassay (Lequin et al. 1970; Schopman et al. 1970) and serum calcium by complexometric titration. In 77 healthy subjects the serum PTH values varied from undetectable (<25) to 415 pg bovine parathyroid hormone equivalent per ml. The limit of detection of the assay is 2.5 pg eq. per 100 µl in the assay. Parallelism with the standard curve of bovine hormone was studied and observed in 5 hyper-reactive cases of PHP. The normal range of serum calcium was 9.0 to 10.6 mg per 100 ml. We examined 8 controls (normal subjects or patients without disturbances of calcium

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metabolism), 28 patients with surgically proven PHP (of whom 5 patients have been re-examined post-operatively) 9 patients with nephrolithiasis and in all but one case (idiopathic) hypercalciuria and 7 patients with hypercalcaemia not caused by parathyroid disease.

The diagnosis and sex and age distribution are given in Table 1. The criteria for the diagnosis of idiopathic hypercalciuria (IHC) were: physical examination essentially normal, hypercalciuria, consistent normocalcaemia; serum electrolytes, proteins and alkaline phosphatase, urinary acidity and radiology of the skeleton, all normal, and histology of an iliac crest bone biopsy (using the Goldner staining of undecalcified bone (Romeis 1968 resp. v. d. Sluys Veer et al. 1964)) normal or showing only moderately accentuated signs of bone resorption and/or formation.

All subjects gave their informed consent to the studies.

RESULTS

As mentioned above, with the assay used previously a normal upper limit of the basal serum IRPTH level of 415 pg bovine hormone equivalent per ml was found. However, in the present study the highest basal values in the control group, the group of IHC (except 1 patient with a level of 1120 pg eq. per

| Table 1. |
| Subjects studied. |

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Control[1]</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>PHP</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Nephrolithiasis (IHC)</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Non-parathyroid hypercalcaemia[2]</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

1) 2 healthy subjects
2) 3 myelomatosis
   2 duod. dulcer
   1 essential hypertension
   1 pulm. embolism
   1 pneumonia
   1 epigastric hernia

   1 mammary carc.  metast. in
   1 anapl. carc.  the skeleton
   1 sarcoidosis
   1 thyrotoxicosis

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ml), the group of patients with PHP successfully operated upon and the group of patients with non-parathyroid hypercalcaemia were 670, 740, 780 and 730 pg eq. per ml respectively (Table 2). Accepting on the basis of these data 780 pg bovine parathyroid hormone eq. per ml as the "normal" upper limit only 7 of the 28 PHP patients studied appeared to have an elevated basal serum IRPTH concentration.

The response of serum PTH to the lowering of calcium EDTA in the control subjects and the patients with PHP is presented semilogarithmically in Fig. 1, A and B. In the majority of cases of PHP the peak level of PTH appeared to be reached at or 30 min before the end of the infusion of EDTA although in several instances it had already been observed 30 min after starting the infusion. In the other groups no special trend as to the timing of the maximal serum PTH concentration was observed. Checking the course of PTH concentration at 30 min intervals for 2 h after completing the EDTA infusion in 26 subjects out of all groups we found a maximal PTH level shortly after the end of the infusion in 3 cases only.

Fig. 2 shows that 22 of the 28 patients with PHP had a maximal increment of PTH (Δ PTH) of 290 pg bovine hormone eq. per ml or more while the upper limit of the increments found in the control group, the group IHC and the group non-parathyroid hypercalcaemia was 270, 245 and 130 pg eq. per ml respectively. For this expression of the PTH release the highest value found in the control group has provisionally been used as the upper limit of the normal. In 13 of the 28 cases of PHP the Δ PTH exceeded 500 pg eq. per ml. The release of PTH in patients suffering from PHP, who were re-examined after surgical intervention and who had exhibited an excessive release of PTH pre-operatively, appeared to be subsequently normalized. Statistical evaluation of these data (Student's t-test) revealed a significant difference between the Δ PTH-values of the hyperparathyroid group and that of each of the other groups (P < 0.005) and no significant differences amongst the Δ PTH-values of these last groups. With regard to the fasting PTH levels in none of the pairs of groups was a significant difference found. It may be seen that the distribution of the Δ PTH-values in the PHP group is rather skewed (Fig. 2). The Δ PTH provided a distinct diagnostic aid in 15 of the 22 hyper-responsive PHP patients in that they combined a basal PTH level of less than 780 pg eq. per ml with a Δ PTH of 290 pg eq. per ml or more.

Expressing the response of the secretion of PTH by the area confined by the basal PTH level and the linear plot of the PTH level with time up to 6 h (i.e. by a summation of PTH levels) did not improve the ability of the test to differentiate between patients with PHP and the other groups.

In view of the differences in response seen within the hyperparathyroid group, the relationship between the maximal decrement of serum calcium (Δ Ca) and the maximal increment of serum IRPTH (Δ PTH) has been studied in this

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Table 2.
Ca and PTH values fasting and during EDTA.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Fasting Ca mg/100 ml</th>
<th>Δ Ca&lt;sup&gt;1)&lt;/sup&gt; mg/100 ml</th>
<th>Fasting PTH pg bov. eq./ml</th>
<th>Δ PTH&lt;sup&gt;1)&lt;/sup&gt; pg bov. eq./ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Controls</td>
<td>8</td>
<td>av. ± sd</td>
<td>9.85 ± 0.48</td>
<td>2.30 ± 0.89</td>
<td>441 ± 196</td>
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<tr>
<td></td>
<td></td>
<td>range</td>
<td>9.24 — 10.52</td>
<td>1.52 — 4.36</td>
<td>130 — 670</td>
</tr>
<tr>
<td>II Nephrolithiasis (IHC)</td>
<td>9</td>
<td>av. ± sd</td>
<td>10.01 ± 0.37</td>
<td>2.06 ± 0.45</td>
<td>432 ± 330</td>
</tr>
<tr>
<td></td>
<td></td>
<td>range</td>
<td>9.20 — 10.44</td>
<td>1.40 — 2.80</td>
<td>25 — 1120</td>
</tr>
<tr>
<td>III Primary hyperparathyroid</td>
<td>28</td>
<td>av. ± sd</td>
<td>11.94 ± 1.05</td>
<td>2.22 ± 0.71</td>
<td>664 ± 449&lt;sup&gt;2)&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>range</td>
<td>10.56 — 14.44</td>
<td>0.72 — 3.80</td>
<td>&lt; 25 — 1790</td>
</tr>
<tr>
<td>IV Primary hyperparathyroid</td>
<td>5</td>
<td>av. ± sd</td>
<td>9.60 ± 0.89</td>
<td>2.06 ± 0.70</td>
<td>490 ± 177</td>
</tr>
<tr>
<td>(post-operatively)</td>
<td></td>
<td>range</td>
<td>8.36 — 10.32</td>
<td>1.12 — 2.80</td>
<td>320 — 780</td>
</tr>
<tr>
<td>V Non-parathyroid hypercalcaemia</td>
<td>7</td>
<td>av. ± sd</td>
<td>11.75 ± 1.65</td>
<td>1.91 ± 1.07</td>
<td>481 ± 187</td>
</tr>
<tr>
<td></td>
<td></td>
<td>range</td>
<td>9.48&lt;sup&gt;4)&lt;/sup&gt; — 13.60</td>
<td>0.56 — 3.60</td>
<td>200 — 780</td>
</tr>
</tbody>
</table>

<sup>1)</sup> Δ Ca: Maximal lowering of the serum calcium concentration by EDTA.
Δ PTH: Maximal increment of the serum parathyroid hormone concentration induced by EDTA.
<sup>2)</sup> Fasting PTH of group III vs. all other groups: n.s.
<sup>3)</sup> Δ PTH of group III vs. those of group I, II and IV: P < 0.005; group III vs. V: P = 0.001 (see text).
<sup>4)</sup> Fasting serum calcium levels of 9.48 and 9.72 mg/100 ml were measured in the tests of the patients with sarcoidosis and thyrotoxicosis 1 and 2 weeks after a hypercalcaemia had been repeatedly found (averages 12.17 and 11.06 mg/100 ml resp.).
Fig. 1.
Semilog plot of the course of serum PTH concentration during and after EDTA.
A: Controls, B: Patients with primary hyperparathyroidism.
group, but no correlation could be detected. A plot of the minimal serum calcium concentration reached against the maximal increment of the PTH level ($\Delta PTH$) during EDTA demonstrated that 14 of the 22 hyper-reactive PHP patients showed a supranormal release of PTH, despite the fact that at no point of the curve was hypocalcaemia observed (Fig. 3). After exclusion of the data of the untreated hyperparathyroid patients an inverse relationship between the minimal serum calcium level and $\Delta$ PTH was observed for the remaining data (Fig. 3).

The PTH release and the amount of parathyroid tissue removed subsequently in 20 cases of PHP (the remaining parathyroid tissue either appearing to be normal in size or equivalent in size to 4 normal parathyroids) appeared to be correlated (Fig. 4). The patients without an elevated $\Delta$ PTH had a relatively small mass of parathyroid tissue (up to about 1 g) subsequently removed. It is also of interest that the patients with enlargement of one gland only (possibly adenomas) did not systematically show a less extensive rise in the PTH level during the EDTA challenge than the patients with multiple enlargement of the parathyroid glands.

Fig. 2.
Maximal increments of the serum PTH levels during EDTA in the 5 groups studied. The means of groups have been indicated.
DISCUSSION

The available data with regard to studies on PTH release in PHP comprise the brief mention of a patient suffering from parathyroid adenoma in whom no release of IRPTH was obtained with infusion of EDTA which lowered the serum calcium from 13.7 to 9.0 mg/100 ml (Buckle 1968) and the negative result reported in a patient with metastatic parathyroid carcinoma despite a decrease in the serum calcium from 16 to below 9 mg/100 ml (Zisman et al. 1968). As already mentioned Potts and coworkers reported that all 20 patients with PHP they investigated by means of infusion of EDTA, showed an increase of 1.5- to 7-fold in the plasma IRPTH concentration with no difference in reaction between the patients with adenomas and those with hyperplasias (Potts et al. 1971; Murray et al. 1972). Recently different mean basal IRPTH levels, mean peak IRPTH and mean response values during EDTA have been reported for 8 control subjects and 6 hyperparathyroid (allegedly adenoma) patients respectively (Wen Chen et al. 1972).

In our hands the best discriminatory parameter for the response of PTH secretion to EDTA in the PHP group as compared to the response in the other
The relationship of the maximal increment of the serum PTH concentration during EDTA in 20 patients with primary hyperparathyroidism and the weight of the parathyroid tissue removed at operation. Between brackets the numbers of glands found to be enlarged.

Groups studied appeared to be the maximal increment in the plasma IRPTH level (Δ PTH) rather than the peak IRPTH value or a summation of PTH levels during infusion of EDTA. Six of the 28 patients with PHP did not show a response of the PTH secretion (Δ PTH) that exceeded the response in the control or the other groups investigated. This lack of response in a minority of the PHP group is not easily explained. Tissue properties with regard to the calcium threshold for PTH secretion may be responsible. The phenomenon may also be caused by different immunochemical properties of the hormone secreted. Up to the present radio-immunoassays of parathyroid hormone are faced with the problem of the varying detection of hormone fragments, circulating after cleavage of the native hormone (Segre et al. 1972).

We do not know whether the assay used in this study detects the native hormone and/or one or more of its cleavage products. Nevertheless, by using this assay in hypercalcaemic patients a supranormal PTH release may be considered diagnostic. In 15 patients with confirmed PHP and basal IRPTH
values not exceeding those in the controls, the diagnosis could be strengthened by a higher than normal PTH release.

Removal of the enlarged parathyroid(s) abolishes an excessive PTH response to EDTA as has also been reported recently by other investigators (Wen Chen et al. 1972).

In connection with the difficult problem of recognizing cases of so-called normocalcaemic PHP in series of patients with nephrolithiasis (Nichols & Flanagan 1967; Wills et al. 1969) we paid special attention to the response of PTH in 9 normocalcaemic patients with calcium containing kidney stones, 8 of whom have been classified as having idiopathic hypercalciuria. They all showed a normal release of PTH, which of course does not exclude the possibility of normocalcaemic PHP occurring in this group.

Specific properties of the assay used must be responsible for the fact that in our small group of patients with non-parathyroid hypercalcaemia no suppression of the basal PTH level, as reported by other investigators (O'Riordan et al. 1972; Murray et al. 1972), has been observed. However, the response of PTH to EDTA appeared to be subnormal to normal in this group.

Theoretically it is significant that 14 of the 22 hyper-reactive patients with PHP showed a high PTH response to EDTA although the serum calcium (measured complexometrically) had been lowered at most to within the normal range. This indicates the occurrence of an elevated calcium threshold of the parathyroid tissue and/or the operation of a non-proportional type of control of the secretion of PTH in these cases. In this connection it is also of interest that in 22 subjects out of the various groups (of whom 13 were hyperparathyroid) the peak PTH level was observed at least 30 min before the minimal serum calcium concentration was reached during the infusion of EDTA.

The correlation between the magnitude of the response of PTH to EDTA and the weight of the pathological parathyroid tissue, referred to by other investigators (Potts et al. 1971), has been established in the present study, although this correlation does not appear to be very close. This is not surprising since it is unlikely that in PHP the essentially abnormal thresholds for suppression and stimulation of the secretion of PTH would have been changed to the same extent from case to case.

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