Regulation of parathyroid hormone release in primary and secondary hyperparathyroidism - studies in vivo and in vitro

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Abstract. The effects of calcium on parathyroid hormone (PTH) release were studied in vivo and in vitro in primary hyperparathyroidism (HPT) and in secondary HPT. In vivo the serum PTH was clearly reduced by intravenous calcium infusion in all the examined patients with primary HPT caused by adenoma. In vitro the release of PTH from dispersed parathyroid cells was likewise suppressed by raising the calcium concentrations in the incubation media, though in all cases a basal release of PTH still persisted even at high calcium concentrations. The degree of suppressibility in vitro varied, but in both primary HPT with adenoma and in secondary HPT it was inversely related to the patients' serum calcium values. These results suggest that the secretion of PTH in patients with primary and secondary HPT is not autonomous either in vivo or in vitro. Furthermore, the non-suppressible basal release of PTH indicates that a major cause for the increased secretion of PTH is the increased number of parenchymal cells. However, the degree of suppressibility of the individual cells rather than the absolute number of cells, seems to be of great importance for the individual serum calcium values in HPT.

Hyperparathyroidism (HPT) is characterized by a disproportionately high secretion of parathyroid hormone (PTH) in relation to the serum calcium level. The pathophysiological background for this condition is still unclear. It has been commonly believed that in the case of parathyroid adenomas the adenoma cells have lost their regulatory capacity and that the secretion of PTH is more or less autonomous. The hypersecretion of PTH in primary or secondary hyperplasia is on the other hand thought to be more normally regulated and caused by the increased glandular mass of otherwise normal cells (Reiss & Canterbury 1969; Mahaffey & Potts 1979). This distinct differentiation between adenomas and hyperplasia can be questioned, not least in view of the uncertainty in the histopathological diagnosis in many cases. It has been suggested, on the basis of histopathological observations, that parathyroid adenomas and hyperplasias are not distinct entities but may both be the result of a stimulation causing first a hyperplasia and later an adenoma (Paloyan et al. 1973).

Knowledge about the morphological and pathophysiological background seems to be of great importance not only for the basic understanding of the pathogenesis of HPT, but also for therapeutic decisions.

The aim of the present study was therefore to investigate the regulation of PTH secretion in primary and secondary HPT by a combination of in vivo and in vitro studies.

Material and Methods

The investigations were made on 41 patients operated on for HPT. Thirty-four of them had primary HPT (21 females, 13 males, mean age 59 years, range 24–82). In all these 34 patients the histopathological diagnosis was adenoma. and the weight of the adenoma as determined
by the pathologist varied between 110 and 8800 mg. Seven patients (4 females, 3 males, mean age 53 years, range 39–59) had HPT secondary to chronic renal failure and all but one were hypercalcaemic prior to surgery. The histopathological diagnosis in these cases was chief cell hyperplasia of varying degree. The combined weight of the 3½–4 extirpated glands varied between 800 and 2400 mg.

**In vivo studies**

In 21 patients with primary HPT and a serum calcium level below 3 mmol/l, a 2 h iv calcium infusion was given with 10 mg of calcium gluconate/kg body weight (Calcium Sandoz) dissolved in 500 ml of saline. The mean serum calcium before the infusion was 2.86 ± 0.11 (SD) mmol/l and at the end of the infusion 3.56 ± 0.25 (SD) mmol/l. The serum PTH concentration was measured at the beginning and at the end of the infusion.

**In vitro studies**

Parathyroid glands from 24 patients with HPT were investigated. Studies were made of adenomatous tissue from 17 patients with primary HPT and of parathyroid tissue from 7 patients with hyperplasia secondary to uraemia.

One normal parathyroid gland accidentally removed at an operation for a goitre was also investigated. Dispersed parathyroid cells were prepared from tissue removed at surgery. The release of PTH from the parathyroid cells was then studied at varying calcium concentrations.

**Cell dispersion**

Parathyroid tissue removed at surgery was immediately minced with scissors in cold tissue culture medium (modified Ham’s F10 without bicarbonate, with 20 mM HEPES (N-2-hydroxyethyl-piperazone-N-2-ethane sulphonic acid), pH 7.45, containing 1 mM CaCl2 and 0.5 mM MgSO4. Dispersed parathyroid cells were obtained by a modification of the technique described by Brown et al. (1978). Pre-incubation with EGTA-buffer A (8.3 g NaCl, 0.5 g KCl, 2.4 g HEPES, H2O to 1 litre, pH adjusted to 7.40 with 1 M NaOH) for 30 min and immediately thereafter digestion of the tissue in 10–20 ml/per gram tissue of buffer B (8.0 g NaCl, 0.35 g KCl, 0.16 g MgSO4·7 H2O, 0.18 g CaCl2·2 H2O, 2.4 g HEPES to 1 litre, pH adjusted to 7.40 with 1 M NaOH) containing 0.2% collagenase (Worthington type I), 40 μg DNase (Sigma type II) per ml and 1.5% bovine serum albumin (Sigma) in a shaking incubator at 37°C for 90 min with aspiration into a 10 ml plastic pipette every 10 min for further disruption of the tissue. Finally the cell suspensions were passed through 125 and 25 μm nylon meshes (Züricher Beutelfabrik AG) and then cleared of most dead cells and debris by layering 2 ml of the cell suspensions dissolved in buffer A on top of 2 ml buffer B containing 1.5% albumin, and then centrifuging at 1000 r.p.m. for 10 min. Microscopy of parathyroid cells was performed with cells in suspension. The viability of the cells was estimated from the capacity of the cells to exclude 0.25% trypan blue. The number of cells was counted in a Burker chamber after dilution with Turk’s reagent.

**Incubation of cells at varying calcium concentrations**

Dispersed parathyroid cells were incubated for 2 h at 37°C in modified F10 tissue culture medium. Plastic tissue culture plates (24 × 2 ml vials/plate, Costar, Cambridge, Mass., USA) were used. Each vial was filled with 0.5 ml F10 medium and 5 × 10⁴ cells. CaCl2 was added to the F10 medium to obtain calcium concentrations of 0.5, 1.0, 2.0 and 3.0 mmol/l, respectively. The protein concentration was low (0.2% ovalbumin) and therefore the calcium in the incubation medium was presumably almost completely ionized. Duplicate incubations at each calcium concentration were carried out and mean values were used for the calculations. The incubation was stopped by pelleting the cells in a Beckman micro-centrifuge at 10 000 r.p.m. for 1 min.

![Graph: Effect of a 2 h calcium infusion on the serum concentration of parathyroid hormone (PTH) in patients with primary hyperparathyroidism. Values after infusion are expressed in per cent of the initial values.](image-url)
**Assays of PTH**

The immunoreactive concentrations of PTH were measured by a radioimmunoassay (Arnaud et al. 1971) employing 125I-labelled bovine PTH (Inolex) and sheep antisera against bovine and porcine PTH (Hehrmann et al. 1980). For the in vivo measurements an antiserum (S478) which measured intact human PTH and the C-terminal two-thirds of the molecule was used. This antiserum reacts predominantly with a mid-portion (44–68) fragment. The reference range, as estimated from 50 healthy individuals, was 0.40–1.20 arbitrary units per litre. For the in vitro measurements another antiserum (S469) was employed, which also primarily recognises the 44–68 fragment of the PTH molecule but has a somewhat lower affinity for the intact hormone. Both antisera were provided by Drs. Herhmann and Hesch, Hannover, West Germany.

**Results**

**In vivo studies**

There was a positive correlation between the pre-operative levels of serum PTH and the weight of the extirpated parathyroid tissue in patients with primary HPT ($r = 0.31, P < 0.001$). No relationship was found, on the other hand, between serum PTH and pre-operative serum calcium. Furthermore serum calcium and the weights of the extirpated parathyroid tissue were not significantly correlated. During calcium infusion all patients with primary HPT and adenoma except one, reacted with reduced serum PTH, in some cases down to 40% of the initial values (Fig. 1). The relative reduction of serum PTH during calcium infusion...
in vivo was not correlated to either the serum calcium level, the initial serum PTH level or the weight of the extirpated parathyroid tissue.

**In vitro studies**

The yield of parathyroid cells obtained with the collagenase treatment was approximately $10^8$ cells/g wet weight. Usually more than 95% of isolated cells were viable as judged from the exclusion of trypan blue. When cells from the parathyroid adenomas were incubated with calcium at varying concentrations the greatest release of PTH was generally noted at the lowest calcium concentration (0.5 mmol/l). The release of PTH was increasingly suppressed by rising calcium concentrations and the lowest values of PTH in the incubation medium were noted at the highest concentration of calcium (3.0 mmol/l). Cells from all adenomas showed a varying degree of suppressibility, with PTH values at the highest calcium concentration ranging from 94 to 40% of the values at the lowest calcium concentration, i.e. in all instances a basal release of PTH remained (Fig. 2a). The PTH value at 0.5 mmol/l was taken as 100%.

The cells from patients with uraemic hyperplasia (Fig. 2b) reacted in a similar way. There was increasing suppression with rising calcium levels and a remaining non-suppressible basal release.

The cells from the investigated normal gland showed a pronounced suppressibility (Fig. 2a,b).

When all patients were considered together, a negative relationship appeared between the individual serum calcium concentrations and the maximum suppressibility in vitro (Fig. 3, $r = -0.45$, $P < 0.05$). Thus the greatest reduction of PTH levels in vitro was found for cells from patients with the lowest serum calcium values.

**Discussion**

The question whether HPT results from an increased number of normal cells or from an overgrowth of a neoplastic cell population that has lost its sensitivity to regulation has been under considerable debate (Mahaffey & Potts 1979). Calcium infusions have been given in investigations in vivo in an attempt to resolve this matter, with somewhat conflicting results. Some investigators have found that PTH levels in patients with HPT are in general not reduced by calcium infusion (Reiss & Canterbury 1969; Gillquist et al. 1979). Most other studies, however, have revealed a suppressibility of PTH secretion during calcium infusions in patients with primary HPT, whether this was caused by parathyroid adenoma or by hyperplasia (Murray et
al. 1972; Bouillon & De Moor 1977; Monchik et al. 1977). Retained regulation of PTH secretion has also been demonstrated in studies on patients with renal failure and secondary HPT (Buckle 1970; Gennuth et al. 1970; Goldsmith et al. 1973; Mahaffey & Potts 1979).

In the present investigation the PTH level decreased in virtually all patients with primary HPT during calcium infusion, in some cases down to as little as 40% of the initial value. There was a wide variation in this respect, but the results strongly suggest that in patients with mild to moderate HPT (serum calcium below 3 mmol/l), the parathyroid hormone secretion is not autonomous.

The interpretation of in vivo studies may be questioned, in view of the possible influence of parathyroid hormone fragmentation and differences in rates of hormonal metabolism on assays of the hormone in peripheral blood (Hehrmann et al. 1980). Furthermore, the question whether the changes in PTH secretion during a calcium infusion test constitute a direct response to the raised calcium concentration or are indirect effects of other hormones remains unanswered. The normal glands associated with an adenoma may also be responsible for some of the hormone measured during a calcium infusion test. It was therefore of considerable interest to find in the present study that also in vitro almost all investigated glands responded to variations in the calcium concentration, irrespective of the patients having primary or secondary HPT. In spite of slight differences in the technique used to prepare dispersed parathyroid cells, the results in this respect are similar to those obtained by Brown et al (1978).

Both in our study and the latter study a non-suppressible basal hormone secretion was demonstrated. The observed non-suppressible basal levels of hormone release indicate that a major factor of importance for the hypersecretion of PTH in HPT is the increase in the number of parenchymal cells. This is supported by the positive correlation observed between the PTH level and the weight of the extirpated parathyroid tissue.

Other evidence supporting this theory has been presented by Gittes & Radde (1966), who produced hypercalcaemia by transplanting multiple normal parathyroid glands into a single animal of an isologous strain of rats. However, from the present findings it seems as if the individual level of serum calcium in patients with both primary and secondary HPT is mainly the result of an impaired regulation of hormone release from the parathyroid cells, as a correlation was demonstrated between serum calcium and suppressibility of the dispersed cells in vitro. Thus, for the individual serum calcium level the degree of suppressibility of the parathyroid cells appeared to be of greater importance than the absolute number of cells i.e. the glandular weight. In accordance with these findings there was no correlation between the weight of the extirpated parathyroid tissue and the serum calcium concentration. Therefore, there seems to be at least two essential factors associated with the regulation of serum calcium in primary HPT, namely the absolute number of the parenchymal cells and the degree of suppressibility of the individual cells. The findings in the present study indicate that a long-term stimulation of the parenchymal cells as in HPT secondary to uraemia may not only increase the number of parenchymal cells but may also affect the cellular regulation of hormone release, which in these patients may be reflected clinically as hypercalcaemia.

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References


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