Parathyroid hormone release in vitro in hyperparathyroidism associated with multiple endocrine neoplasia type 1

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Abstract. Hyperparathyroidism (HPT) in the syndrome of multiple endocrine neoplasia type 1 (MEN-1) exhibits a different picture regarding its propensity for recurrence compared with sporadic primary HPT. In order to shed further light on the MEN-1 syndrome an investigation in vitro was made of parathyroid hormone (PTH) release of dispersed parathyroid cell from 11 patients with parathyroid hyperplasia associated with MEN-1, 10 patients with single parathyroid adenomas, and 10 preparations of normal bovine parathyroid glands. The two patient groups had the same average serum calcium value prior to surgery. Immunoreactive concentrations of PTH were measured after 2-h incubations at extracellular calcium concentrations of 0.5–3.0 mmol/l. Compared with the normal bovine parathyroid cells, the cells of the MEN-1 patients had a reduced calcium sensitivity of the PTH release and secreted smaller amounts of hormone at both low and high extracellular calcium concentrations. A similar abnormality of the PTH release was found for the cells of the hyperplastic and adenomatous parathyroid glands. Although individual parathyroid glands were investigated in only three MEN-1 patients, the results suggested the secretory regulation to be less defective in the small glands of each patient. It is concluded that in patient groups matched for serum calcium, the parathyroid tissue of MEN-1 patients has an abnormality of the PTH release similar to that of parathyroid adenomas.

Primary hyperparathyroidism (HPT) of the multiple endocrine neoplasia syndrome type 1 (MEN-1) is characterized by hyperplasia of the parathyroid glands with asymmetrical glandular enlargement (Yamaguchi et al. 1980; Allo & Thompson 1983). In vitro studies of parathyroid cells from HPT patients with the MEN-1 syndrome are scarce and the occasional patients investigated have shown a parathyroid hormone (PTH) release with an almost normal responsiveness to changes in the ambient calcium concentration (Brown et al. 1978, 1979). In contrast, dispersed cells of parathyroid adenomas generally have a reduced sensitivity to extracellular calcium (Brown et al. 1978, 1979; Rudberg et al. 1982). It has also been shown that the degree of calcium sensitivity of the PTH release seems to determine the serum calcium value of hypercalcaemic patients with adenomatous HPT and HPT secondary to renal insufficiency (Rudberg et al. 1982).

The present experimental study was performed to clarify the pattern of PTH release in the primary parathyroid hyperplasia of the MEN-1 syndrome and to elucidate a possible pathophysiological difference in hormone regulation as compared with parathyroid adenomas. For the purpose of attaining as closely comparable patient groups as possible, the patients with adenomatous HPT were selected so as to obtain a level of serum calcium similar to that in the MEN-1 patients.

Methods

Parathyroid tissue was obtained during total parathyroidectomy and autotransplantation of 11 patients with primary HPT associated with the MEN-1 syndrome.
The pre-operative serum calcium values were 2.64–3.22 mmol/l (normal range 2.20–2.60 mmol/l, Table 1). Eight of the patients were subjected to primary operations, and four glands were identified and removed in each of them. The glandular size in the parathyroid tissue was 200–2100 mg (mean 940 mg). One gland was extirpated at re-operation in 3 other patients, and the weights of these glands varied between 200 and 800 mg (mean 400 mg). Histopathological examination substantiated the diagnosis of parathyroid hyperplasia in all the patients. After autotransplantation to the forearm of approximately 60 mg of parathyroid tissue (cf Malmaeus et al. 1986), all remaining tissue from each MEN-1 patient was handled together in the investigation of dispersed cells except in 3 patients in whom two-three glands were treated separately.

Single parathyroid adenomas were obtained from 10 patients with serum calcium values from 2.62–3.10 mmol/l (Table 1). The adenomas weighed from 250–1200 mg (mean 680 mg). Bovine parathyroid glands were removed on 10 occasions from adult cattle immediately after slaughtering and used as normal reference (cf Larsson et al. 1984).

After conventional histopathological examination, the available parathyroid tissue was thoroughly minced with scissors in cold tissue culture medium (modified Ham’s F10 with 20 mmol/l Hepes, pH 7.4 and 1.25 mmol/l Ca²⁺). The erythrocytes and fat cells were crudely removed by two washings and centrifugation (500 rpm, 2–3 min) in buffer 1 (20 mmol/l Hepes, 136 mmol/l NaCl, 4.7 mmol/l KCl, 0.65 mmol/l MgSO₄ and 1.2 mmol/l CaCl₂, pH 7.45) containing 0.1% (w/v) bovine serum albumin (Sigma, Fraction V). The tissue was digested for 30–45 min with collagenase (Boehringer Mannheim, 2 mg/ml) and DNase (Sigma DN-25, 0.05 mg/ml) in buffer 1 containing 1.5% (w/v) bovine serum albumin, by the use of a shaking incubator (250 c/min, 37°C) and aspirations with a Pasteur pipette. The cell suspension was filtered through nylon meshes (28 μm), spun down (1000 rpm, 10 min) and subjected to a short rinse in 1 mM EGTA in buffer 2 (20 mmol/l Hepes, 142 mmol/l CaCl₂, and 6.7 mmol/l KCl, pH 7.4). Dead cells and debris were removed by centrifugation (1000 rpm, 10 min) on a discontinuous Percoll gradient (Pharmacia Fine Chemicals) of 25% standard isotonic Percoll (Rudberg et al. 1986). The cells were removed from the surface of the gradient and washed twice in buffer 1.

The dispersed cells were counted in a Bürker chamber after dilution with Türk’s reagent. Approximately 10⁸ cells were gained per g of parathyroid tissue. More than 95% of the cells were viable as judged by Trypan blue exclusion. The cells were pre-incubated for 30 min at 37°C in a 20 mmol/l Hepes-buffer containing 1.25 mmol/l CaCl₂ and physiologically balanced in respect to other cations with Cl⁻ as the only anion (Hellman 1975).

PTH release was determined in duplicate incubations of 5 × 10⁶ parathyroid cells during 2 h in 0.5 ml of the buffer of Hellman (1975) containing 0.5–3.0 mmol/l Ca²⁺ and 0.2% (w/v) human serum albumin (Behring Institute). The suppressibility of the PTH release was calculated as the relative reduction in PTH between ambient calcium concentrations of 0.5 and 3.0 mmol/l.

The immunoreactive concentrations of PTH in the incubation medium were measured by a radioimmunoassay employing 125I-labelled bovine PTH (Inolex) and sheep antiserum (S-469) against bovine and porcine PTH primarily recognizing the 44–68 fragment of the PTH molecule and with a somewhat lower affinity for the intact hormone (Hehrmann et al. 1980). In each cell preparation, the variation in PTH values was less than 10% between the duplicate incubations at each calcium concentration. All PTH measurements were made at an interval of the standard curve where human and bovine PTH were found to dilute in parallel. The PTH values in vivo were determined by a similar assay system.

<table>
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<tr>
<th></th>
<th>MEN-1</th>
<th>Adenoma</th>
<th>Bovine cells</th>
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<tbody>
<tr>
<td>Serum calcium (mmol/l)</td>
<td>2.83 (0.04)</td>
<td>2.83 (0.03)</td>
<td>–</td>
</tr>
<tr>
<td>Serum PTH (arbU/l)</td>
<td>1.15 (0.06)</td>
<td>1.04 (0.10)</td>
<td>–</td>
</tr>
<tr>
<td>PTH release (ng/2 h/10⁵ cells)</td>
<td>0.5 mmol/l 9.2 (3.1)*</td>
<td>16.3 (5.2)</td>
<td>41.2 (6.1)</td>
</tr>
<tr>
<td></td>
<td>3.0 mmol/l 5.6 (2.1)*</td>
<td>9.6 (5.3)</td>
<td>16.7 (3.7)</td>
</tr>
<tr>
<td>Supressibility (%)</td>
<td>39 (7.1)*</td>
<td>44 (6.3)</td>
<td>61 (3.9)</td>
</tr>
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</table>

Table 1. Mean pre-operative serum calcium and PTH values for the patients with MEN-1 and adenomatous HPT. Mean suppressibility and amounts of PTH released at 0.5 and 3.0 mmol/l Ca²⁺ for the two patient groups and for 10 experiments with bovine parathyroid glands. SEM in parenthesis. *P < 0.01, refers to comparison with the normal bovine cells.
employing another sheep antiserum (S-478) (Hehrmann et al. 1980).

A non-parametric test of significance (Wilcoxon rank sum test) was applied to evaluate the difference between the MEN-1 and adenoma patients with respect to pre-operative serum calcium and PTH values. The same test was also used to compare the preparations of hyperplastic glands with those of adenomatous and normal bovine glands with respect to the suppressibility of the PTH release and the amounts of PTH released at calcium concentrations of 0.5 and 3.0 mmol/l. Regression analysis was used to correlate the pre-operative serum PTH values with the weights of the abnormal parathyroid tissue in the MEN-1 and adenoma patients. A probability of less than 5% was accepted as statistically significant.

Results

The mean pre-operative serum calcium and PTH values of the MEN-1 patients were similar to those of the adenoma patients (Table 1). The PTH

![Fig 1](image-url)

**Fig. 1.**
Total glandular weight and preoperative serum PTH values in the MEN-1 (N = 10, ○) and adenoma cases (N = 10, ●). The regression line for the adenomas (r = 0.88) is shown.

![Fig 2](image-url)

**Fig. 2.**
Relative PTH release in relation to extracellular calcium in parathyroid cells from 8 MEN-1 patients (left) and 10 adenomas (right). The average values (± SEM) for 10 experiments with normal bovine cells are indicated by the dotted lines.
values were not significantly correlated to the weight of the abnormal parathyroid tissue in the MEN-1 patients, but a correlation \( r = 0.88, P < 0.001 \) was found in adenoma patients (Fig. 1).

The parathyroid cells of the hyperplastic glands from the MEN-1 patients had an average PTH release of 9.2 and 5.6 ng/2 h/10^5 cells at 0.5 and 3.0 mmol/l calcium, respectively (Table 1). The corresponding release for the adenoma preparations was 16.3 and 9.6 ng (differences not statistically significant). Compared with the bovine cells, the cell suspensions of the individual HPT patients released a more variable amount of PTH. The PTH release of the cells from the hyperplasias was significantly smaller \( P < 0.01 \) than that of the bovine cells at both the low (0.5 mmol/l) and high (3.0 mmol/l) extracellular calcium concentrations.

The PTH release of the cells from the hyperplastic and adenomatous glands showed a similarly heterogeneous sensitivity to extracellular calcium (Fig. 2). The average suppressibility of the parathyroid cells of the MEN-1 patients was 39% which was not statistically different from that of the adenomas, but significantly smaller \( P < 0.01 \) than that of the normal bovine cells (Table 1). With few exceptions, the preparations of abnormal glands displayed a right-shift of the curve relating PTH release to extracellular calcium (Fig. 2). There was no difference in this respect between the cells of hyperplastic and adenomatous glands.

When individual glands from the same MEN-1 patient were compared (Fig. 3), the cells of larger glands displayed less suppression of the PTH release and greater right-shift of the calcium-PTH curve. A normal-sized gland of one MEN-1 patient showed a responsiveness similar to that of the normal bovine cells (Fig. 3A).

Discussion

HTP is seldom of MEN-1 origin (Mallette et al. 1974; Muhr et al. 1984), but the expressed syndrome is frequently and perhaps even invariably associated with HPT, which is generally the primary manifestation of the disorder (Ballard et al. 1964). Apart from being an autosomal dominant trait (Wermer 1954; Steiner et al. 1968), the HPT of the MEN-1 syndrome is characterized by hyperplasia with a pronounced rate of recurrence after parathyroid surgery (Wells et al. 1980; Allo & Thompson 1983; van Heerden et al. 1983; Rizzoli et al. 1985). This may depend on genetically determined local or systemic factors stimulating the growth of the parathyroid tissue.

The present investigation revealed a defective regulation of the PTH release from the parathyroid cells of HPT patients with the MEN-1 syndrome. In comparison with the normal bovine cells, the parathyroid tissue of these patients released smaller amounts of PTH with a reduced

![Fig. 3.](image-url) Relative PTH release from individual glands of 3 MEN-1 patients (A–C). Glandular weights are depicted.

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suppressibility in response to increases in the ambient calcium concentration and a right-shift in the curve relating PTH to calcium. It has previously been reported that the hyperplastic parathyroid tissue of MEN-1 patients retains an almost normal responsiveness to ambient calcium (Brown et al. 1978, 1979). However, in their studies three out of four MEN-1 patients had serum calcium values within the normal range, whereas all our patients were hypercalcaemic.

In corroboration of previous studies (Brown et al. 1978, 1979, 1981; Rudberg et al. 1982), the PTH release of dispersed adenoma cells had an abnormally low calcium sensitivity, and the amounts of hormone released were reduced in relation to the normal bovine parathyroid cells.

The material of the present study was selected to obtain a similar level of serum calcium within the groups of patients with adenomatous and MEN associated HPT. The analysis revealed that the MEN-1 patients had a similarly deranged regulation of the PTH release as the patients with single parathyroid adenomas. Although measurements of immunoreactive PTH in the incubation medium may be insufficient for studies of abnormal parathyroid cells owing to the release of PTH fragments (Habener 1978), the results suggest that the secretory regulation rather than the capacity of the PTH release determines the serum calcium value in patients with parathyroid hyperplasia of the MEN-1 syndrome just as previously found in patients with parathyroid adenomas (Rudberg et al. 1982).

A different abnormality of the PTH release was found between the individual glands of three MEN-1 patients, the smaller glands appearing to have a less deranged hormone release. Although only few patients were investigated in this respect, the results may suggest that tissue excised for parathyroid autotransplantation in MEN-1 patients should preferably be selected from the smallest glands as they may exhibit the least disturbed hormone regulation of the parathyroid tissue from each patients.

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References


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