Calcium-Sensing Receptor Expression and Parathyroid Hormone Secretion in Hyperplastic Parathyroid Glands from Humans

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In uremic patients, severe parathyroid hyperplasia is associated with reduced parathyroid calcium-sensing receptor (CaR) expression. Thus, in these patients, a high serum Ca concentration may be required to inhibit parathyroid hormone (PTH) secretion. This study compares the magnitude of reduction in CaR expression and the degree of the abnormality in Ca-regulated PTH release in vitro. A total of 50 glands from 23 hemodialysis patients with refractory hyperparathyroidism were studied. Tissue slices were incubated in vitro to evaluate (1) the PTH secretory output in a normal Ca concentration (1.25 mM) and (2) the PTH secretory response to high (1.5 mM) and low (0.6 mM) Ca concentration. Tissue aliquots were processed for determination of CaRmRNA expression. The results showed that, corrected for DNA, parathyroid tissue with lowest CaR expression secreted more PTH than that with relatively high CaR expression (146 ± 23 versus 60 ± 2 pg/μg DNA; P < 0.01). Furthermore, glands with low CaR expression demonstrated a blunted PTH secretory response to both the inhibitory effect of high Ca and the stimulatory effect of low Ca. The study also showed that the larger the gland, the lower the CaRmRNA expression. Thus, large parathyroid glands produce a large amount of PTH not only as a result of the increased gland size but also because the parathyroid tissue secretory output is increased. These abnormalities in PTH regulation are related to low CaR expression.


The parathyroid calcium-sensing receptor (CaR) enables the parathyroid cell to respond to changes in extracellular Ca concentration. Activation of parathyroid CaR by high extracellular Ca results in reduction of parathyroid hormone (PTH) secretion, whereas low extracellular Ca deactivates the CaR, promoting the release of PTH (1).

Patients with chronic kidney disease have an alteration in mineral metabolism that results in progressive parathyroid gland hyperplasia (2). In dialysis patients, the parathyroid glands may become very large with the presence of nodules as a result of monoclonal cell proliferation (3,4). These patients may require parathyroidectomy because they do not respond adequately to the standard medical treatment of hyperparathyroidism: control of phosphate and administration of vitamin D (5). The lack of response to medical treatment may be explained by a decrease in the expression of parathyroid vitamin D receptors (VDR) and CaR in severe parathyroid hyperplasia (6–10).

Some authors have observed that in advanced parathyroid hyperplasia, the sensitivity of the parathyroid cell to Ca is decreased and a high serum Ca concentration is required to inhibit PTH secretion. Thus, the PTH-Ca curve is shifted to the right and there is an increase in the setpoint of Ca for PTH secretion (11,12). This has been attributed to a reduction in CaR expression observed in parathyroid glands with advanced hyperplasia (9,10). This issue can be addressed directly by comparing the magnitude of reduction in CaR expression with the degree of the abnormality in Ca-regulated PTH release in vitro.

In healthy adults with normal serum Ca concentration, the parathyroid glands’ secretory rate is at 25% of their maximal capacity (13). However, in hyperplastic glands with low CaR expression, a normal serum Ca concentration may not be able to maintain the basal PTH secretion reduced to a rate as low as 25% of maximal. Previous publications have shown that in patients with GFR <30 ml/min, basal PTH secretion relative to its maximal capacity is significantly increased as compared with control subjects (46 versus 27%), and in dialysis patients, this value is even higher (52%) despite normal serum Ca concentration (14,15). In this study, we assessed whether for an
equal amount of hyperplastic parathyroid tissue the production of PTH is affected by the level of CaR expression. Thus, the aim of this study was to determine in vitro the effect of CaR expression on the regulation of PTH secretion by Ca in human hyperplastic parathyroid glands.

Materials and Methods
The study was performed in freshly excised parathyroid glands from hemodialysis patients who had hyperparathyroidism and underwent parathyroidectomy because they failed to respond to medical treatment.

Parathyroid Tissue
A total of 30 glands from 23 patients (male/female, 12/11) were evaluated. Immediately after parathyroidectomy, parathyroid gland tissue was placed at 4°C in RPMI-164 medium with 1.5 mM Ca and experiments were performed 14 to 16 h later. Before the experiment, all parathyroid tissue available was cut into small slices of approximately 1 mm³ to maintain tissue architecture and to be able to perform tests in vitro. Representative aliquots were used for evaluation of gene expression and PTH secretion in vitro. Thirty-two parathyroid glands were processed for RNA extraction and the subsequent measurement of CaR/actin and VDR/actin-mRNA. Each parathyroid gland was treated as one unit: Five to 10 slices were separated at random and mixed. In most glands (27 of 32), the amount of tissue was abundant and 10 slices were available for mRNA measurement. The same slices were used to assess regulation of PTH secretion by high and low Ca. In each gland, the experiment was repeated two to four times (depending on the amount of tissue available) using 10 separate slices in each experiment. We found excellent reproducibility in the results obtained using different sets of aliquots from the same gland. This suggests that the set of 10 slices was representative of the entire gland. An additional group of 18 parathyroid glands were used to evaluate the PTH-Ca curve in vitro and the CaRmRNA expression; this experiment was performed once per gland using 10 slices of parathyroid tissue, and an aliquot of 10 slices was used to measure CaR/actin-mRNA.

Incubation Conditions
Parathyroid tissue slices were placed resting inside a nylon basket in individual wells (24-well dishes; Nuclon Delta SI, InterMed, Roskilde, Denmark) with constant shaking at 37°C in a humid atmosphere. The incubation medium was buffered (7.4) and contained (in mM) 125 NaCl, 5.9 KCl, 0.5 MgCl₂, 1 mM phosphate (NaH₂PO₄: Na₂HPO₄, 1:2 ratio), 1 Na pyruvate, 4 glutamine, 12 glucose, and 25 HEPES. Insulin concentration measured in the supernatant was the baseline PTH secretion.

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Determination of CaRmRNA and VDRmRNA
Total RNA was extracted following a modification of Chomczynski and Sacchi’s protocol (16). The RNA was dissolved in nuclease-free water (Promega, Madison, WI) and heated for 10 min at 60°C. Total RNA was quantified by spectrophotometry (17). CaR versus actin and VDR versus actin were amplified using the kit Access RT-PCR System (Promega) using specific primers for β-actin (5’-Hex CGTCACCAA-CTGGAGCACATGGAG-3’, 5’-GGCTGTCAGGATGACCCACAGGCCTGGA-3’), VDR (5’-6-Fam TGAACGCACAAAGCGTCACCCGGG-3’, 5’-GGATGAACCTCTCTACATGCCGATG-3’) and CaR (5’-Hex ATTGAGGGAGCCCACTGTC-3’, 5’-AAAGAGGTGAGTCCGATCCAAAAG-3’) sense and antisense, respectively (Tip molbiol, Berlin, Germany). One hundred nanograms of total RNA was used per sample. DNA amplifications were processed by a Genetic Analyzer ABI Prism 310 (Perkin Elmer, Foster City, CA). Data were analyzed using a specific software Gene Scan v 3.1/1998 (Perkin Elmer).

Determination of CaR Protein
The relationship between CaRmRNA expression and the amount of CaR protein was assessed by measuring both mRNA and protein in the same parathyroid sample. Tissue from glands was homogenized in ice-cold lysis buffer (50 mmol/L Tris-HCl [pH 7.4], 0.25 mol/L sucrose, 1 mM EGTA and 1 mM EDTA, and 10 µg/ml protease inhibitor). Nuclei and cell debris were removed by low-speed centrifugation (800 × g). The supernatant then was sedimented at 45,000 × g for 1 h, and the pellet was solubilized with 1% Triton X-100 in 100 µl of lysis buffer and stored at −20°C until protein quantification. Proteins (25 µg) were separated by SDS-PAGE (NuPAGE 7% Tris-Acetate gel, Invitrogen/Life Technologies) and transferred (Bio-Rad Transfer System; Hercules, CA) to nitrocellulose filters (Invitrogen/Life Technologies). After incubation for 2 h with TBBS-L blocking solution (Tris-HCl [pH 7.6], Tween 20 0.2%, NaCl 150 mM, and 5% dry milk) at room temperature, filters were incubated overnight at 4°C with 1:2000 solution of anti-CaR polyclonal antibody raised in rabbit. The filters then were re-incubated with anti-rabbit IgG conjugated with alkaline phosphatase at room temperature for 2 h. The signal obtained was quantified using an image-densitometer (GS-800; Bio-Rad) and expressed as intensity absolute value.

Evaluation of PTH Secretion In Vitro
After 6 h of stabilization in incubation medium that contained 1.25 mM Ca, the parathyroid tissue was transferred to fresh medium that contained 1.25 mM Ca and incubated for an additional hour. The PTH concentration measured in the supernatant was the baseline PTH secretion. Thereafter, the same parathyroid tissue was transferred for an additional period of 1 h to low Ca (0.6 mM) and then to high Ca (1.5 mM) to evaluate the inhibition of PTH secretion by Ca. Other tissue aliquots were transferred to 1.5 mM Ca and then to 0.6 mM Ca to evaluate the stimulation of PTH secretion by low Ca. Depending on the amount of tissue available, the experiments were repeated up to four times in the same gland; thus, results obtained in each gland are shown in the figures as mean ± SEM. A separate group of 18 parathyroid glands were used to evaluate in vitro the PTH-Ca curve. Ten tissue slices were sequentially exposed for 1-h periods to Ca concentrations (in mM) of 0.6, 0.8, 1.0, 1.2, 1.5, and 2.5. This experiment was performed once per gland. The setpoint was calculated as the Ca concentration required to decrease maximal PTH secretion to half of the difference between maximal and minimal PTH. Intact PTH secretion was measured in the supernatant using a human Nichols first-generation Intact PTH IRMA (Nichols Institute, San Juan de Capistrano, CA) with intra- and interassay coefficient of variations of 4.3 and 4.7%, respectively.

Statistical Analyses
Comparisons between more than two means were performed by ANOVA. Unpaired t test was used for comparison of two means. Linear regression analysis was used to correlate two variables. Best fit
for nonlinear correlation was determined by Curve Fitting Analysis (SPSS 11.0 for Windows).

Results

A total of 50 glands from 23 patients (male/female, 12/11) were evaluated. Patient age ranged from 23 to 73 yr. The mean time on dialysis was 78 ± 18 mo. At the time of parathyroidectomy, patients had been without calcitriol for 3 to 10 wk. The mean serum intact PTH (Nichols first-generation intact PTH IRMA) Ca and phosphate levels were 1449 ± 234 pg/ml and 10.3 ± 0.5 mg/dl and 6.1 ± 0.7 mg/dl, respectively.

The relationship between the secretion of PTH by human hyperplastic parathyroid tissue slices in vitro and the expression of CaRmRNA expression is shown in Figure 1. In a medium with a normal Ca concentration of 1.25 mM, the PTH secretion by each parathyroid gland was inversely correlated to the amount of CaRmRNA. As shown in Figure 1A, the inverse relationship between PTH secretion and CaRmRNA expression was not linear. Curve-fitting analysis revealed that the data points fitted an inverse function curve (18) ($r^2 = 0.348$, $P < 0.001$). The CaR/actin-mRNA ranged from near 0 to 12. For facilitating comparisons between glands with different levels of expression of CaR/actin-mRNA, glands were divided into three groups according to the level of CaR/actin-mRNA expression: Group I (CaR/actin-mRNA <4), group II (4 ≥ CaR/actin-mRNA < 8), and group III (8 ≥ CaR/actin-mRNA). The rate of PTH secretion in parathyroid glands from group I was greater than in glands from groups II and III (Figure 1B). No statistical difference was observed in the rate of PTH secretion in groups II and III.

Because the secretion of PTH in response to extracellular Ca is mediated by the CaR, parathyroid glands with poor CaR expression may not respond adequately to a change in extracellular Ca. Figure 2A shows the change in PTH secretion in response to an increase in Ca in the medium from 0.6 to 1.5 mM. Changes in PTH secretion that occurred after exposure of tissue to a Ca concentration of 1.5 mM are shown as a percentage of the PTH secreted at the 0.6 mM Ca concentration. The reduction of PTH secretion by Ca was inversely related to the CaR/actin-mRNA expression, and the data points were adjustable to an inverse function curve (18) ($r^2 = 0.309$, $P < 0.001$). Parathyroid glands with relatively high CaR/actin-mRNA (≥4; groups II and III) showed a significant reduction of PTH secretion in response to high Ca. However, glands with CaR/actin-mRNA <4 (group I) did not show a significant inhibitory response to high Ca (Figure 2B). In fact, in some of the glands with low CaR expression, PTH secretion increased despite high Ca in the medium, suggesting that in these glands, PTH secretion was independent of the Ca concentration in the medium.

Changes in PTH secretion in response to a decrease in extracellular Ca (from 1.5 to 0.6 mM) are presented in Figure 3. The changes in PTH secretion after exposure to 0.6 mM extracellular Ca are shown as a percentage of the PTH secreted at the 1.5 mM Ca concentration. The relationship between PTH response to low Ca and the amount of CaR/actin-mRNA is shown in Figure 3A. The data points are best fitted by a negative inverse func-

![Figure 1](image1.png)

**Figure 1.** (A) The relationship between the in vitro basal parathyroid hormone (PTH) secretion (i-PTH, [pg]/[μg DNA]/h) and the calcium-sensing receptor expression (CaR/actin-mRNA) by human hyperplastic parathyroid tissue from patients with uremic hyperparathyroidism. The experiments were performed using tissue slices that were incubated for 1 h in 1.25 mM Ca concentration. Data points are the mean ± SEM of two to four repeated experiments using different tissue aliquots from the same gland. The data points were adjusted to an inverse function curve ($r^2 = 0.348$, $P < 0.001$). (B) Basal PTH secretion (i-PTH, [pg]/[μg DNA]/h) by parathyroid tissue presented in A separated by groups according to the level of CaR/actin-mRNA: Group I (CaR/actin-mRNA <4), group II (4 ≥ CaR/actin-mRNA < 8), and group III (CaR/actin-mRNA ≥8). Bars represent the mean ± SEM. *$P < 0.01$ versus group I. Mean ± SEM (μ) values for groups I, II, and III are 146 ± 23 (21), 85 ± 14 (7), and 60 ± 2 (5), respectively.
tion curve (18) ($r^2 = 0.309, P < 0.001$). Only parathyroid glands from group III (CaR/actin-mRNA ≥ 8) demonstrated a significant increase in PTH secretion in response to low Ca (217 ± 24%). In parathyroid glands with lower CaR expression, exposure to the low Ca-containing medium failed to produce a significant increase in PTH secretion: 121 ± 18 and 153 ± 16%.

Figure 2. (A) The relationship between inhibition of PTH secretion by high extracellular Ca (increase in Ca from 0.6 to 1.5 mM) and CaR/actin-mRNA by human hyperplastic parathyroid tissue from patients with uremic hyperparathyroidism. The experiments were performed using tissue slices that were incubated for 1 h in 0.6 mM and then transferred to 1.5 mM Ca for 1 additional hour. The changes in PTH are presented as the percentage from the PTH secreted at the 0.6 mM Ca concentration. Data points are the mean ± SEM of two to four repeated experiments using different tissue aliquots from the same gland. The data points were adjustable to an inverse function curve (18) ($r^2 = 0.309, P < 0.001$). (B) Inhibition of PTH secretion by high extracellular Ca (increase in Ca from 0.6 to 1.5 mM; expressed as percentage from the PTH secreted at the 0.6-mM Ca) in the same parathyroid tissue presented in A separated according to the level of CaR/actin-mRNA: Group I (CaR/actin-mRNA < 4), group II (4 ≥ CaR/actin-mRNA < 8), and group III (CaR/actin-mRNA ≥ 8). Bars represent the mean ± SEM. *$P < 0.05$ versus group I. Mean ± SEM ($n$) values for groups I, II, and III are 108 ± 7 (21), 70 ± 7 (7), and 56 ± 7 (5), respectively.

Figure 3. (A) The relationship between stimulation of PTH secretion by low extracellular Ca (decrease in Ca from 1.5 to 0.6 mM) and CaR/actin-mRNA by human hyperplastic parathyroid tissue from patients with uremic hyperparathyroidism. The experiments were performed using tissue slices that were incubated for 1 h in 1.5 mM and then transferred to 0.6 mM Ca for 1 additional hour. The changes in PTH are presented as the percentage from the PTH secreted at the 1.5 mM Ca concentration. The data points are best fitted by a negative inverse function curve (18) ($r^2 = 0.308, P < 0.001$). (B) Stimulation of PTH secretion by low extracellular Ca (decrease in Ca from 1.5 to 0.6 mM; expressed as percentage from the PTH secreted at the 1.5 mM Ca concentration) in the same parathyroid tissue presented in A separated according to the level of CaR/actin-mRNA: Group I (CaR/actin-mRNA < 4), group II (4 ≥ CaR/actin-mRNA < 8), and group III (CaR/actin-mRNA ≥ 8). Bars represent the mean ± SEM. **$P < 0.01$ versus groups I and II, respectively. Mean ± SEM ($n$) values for groups I, II, and III are 121 ± 18 (21), 153 ± 16 (7), and 217 ± 25 (5), respectively.
in glands from groups I and II, respectively (Figure 3B). To determine whether the expression of CaR/actin-mRNA reflected the amount of CaR protein, both CaR/actin-mRNA and protein were measured in aliquots from 14 different parathyroid gland samples. Linear regression analysis showed that CaRmRNA correlated significantly with CaR protein ($r^2 = 0.501; P = 0.01$).

The gland’s ability to respond to both high and low Ca concentration was analyzed in relation to the basal PTH secretion rate at a normal Ca concentration (1.25 mM Ca). The relationship between the inhibition of PTH secretion by high Ca and the basal PTH secretion rate is shown in Figure 4. Most glands with basal PTH secretion rate >150 pg/μg DNA/h (which also had low CaR expression) did not reduce PTH secretion in response to a high Ca concentration in the medium. Inhibition of PTH secretion by high Ca was observed only in glands with relatively low basal PTH secretion rates. As shown in Figure 4, most glands with low basal PTH secretion rates had relatively high CaR expression. Similarly, parathyroid glands with high basal rate of PTH secretion did not increase the secretion of PTH in response to low Ca in the medium, whereas glands with relatively low basal PTH secretion (and higher CaR expression) were stimulated by the low Ca in the medium (Figure 5).

The relationship between the setpoint of the PTH-Ca curve and CaR/actin-mRNA expression was analyzed in a separate group of glands, and the results are shown in Figure 6. There was a significant inverse nonlinear correlation (S curve; $r^2 = 0.680, P < 0.01$) between the setpoint and CaR expression. The setpoint is low in glands with relatively high CaR expression (groups II and III). By contrast, in glands with low CaR expression (group I), the setpoint of PTH secretion may be low or high. It is interesting that the relationship between the setpoint and CaR expression is nonlinear, similar to what was observed between PTH inhibition by Ca and CaR expression shown in Figure 2A.

Figure 4. The relationship between inhibition of PTH secretion by high extracellular Ca (increase in Ca from 0.6 to 1.5 mM) and basal PTH secretion at 1.25 mM Ca. The changes in PTH are presented as the percentage from the PTH secreted at the 0.6 mM Ca concentration. Data points are the mean ± SEM of two to four repeated experiments using different tissue aliquots from the same gland. Parathyroid glands were classified according to the level of CaR/actin-mRNA in group I (CaR/actin-mRNA <4), group II (4 ≤ CaR/actin-mRNA < 8), and group III (CaR/actin-mRNA ≥8). The data points are adjusted to a negative inverse function curve ($r^2 = 0.212, P < 0.01$).

Figure 5. The relationship between stimulation of PTH secretion by low extracellular Ca (decrease in Ca from 1.5 to 0.6 mM) and basal PTH secretion at 1.25 mM Ca. The changes in PTH are presented as the percentage from the PTH secreted at the 1.5-mM Ca concentration. Data points are the mean ± SEM of two to four repeated experiments using different tissue aliquots from the same gland. Parathyroid glands were classified according to the level of CaR/actin-mRNA in group I (CaR/actin-mRNA <4), group II (4 ≤ CaR/actin-mRNA < 8), and group III (CaR/actin-mRNA ≥8). The data points are adjusted to an inverse function curve ($r^2 = 0.235, P < 0.01$).

Figure 6. The relationship between setpoint of the PTH-Ca curve and the CaR/actin-mRNA in human hyperplastic parathyroid tissue from patients with uremic hyperparathyroidism. The values of the setpoint were calculated from the individual PTH-Ca curves obtained in vitro. The data points were adjustable to an S function curve (18) ($r^2 = 0.705, P < 0.01$).
The decrease in CaR expression has been associated with severe parathyroid gland hyperplasia. We found that the CaR/actin-mRNA correlated inversely with the gland weight and followed an S curve (18) \((r^2 = 0.150, P < 0.05)\). The overall mean \pm SEM of parathyroid gland weights was 0.92 \pm 0.09 g, with a range of 0.10 to 2.09 g. The respective mean values of parathyroid gland weight in groups I, II, and III were 1.42 \pm 0.26, 0.68 \pm 0.08, and 0.52 \pm 0.10 g; the mean weight of glands from group I was significantly greater than the weight of glands included in this study was 3.8 \pm 0.37 g. The mean \pm SEM of VDR/actin-mRNA of glands included in this study was 3.8 \pm 0.37 and ranged from 0.50 to 8.38. The respective mean values of VDR/actin-mRNA in group I (CaR/actin-mRNA <4), group II (4 \leq CaR/actin-mRNA < 8), and group III (8 \leq CaR/actin-mRNA) were 3.30 \pm 0.38, 4.11 \pm 1.06, and 5.22 \pm 0.97 (NS). Although there seems to be a trend (more CaR is associated with higher VDR expression), correlation analysis between CaR and VDR expression did not reach statistical significance \((r^2 = 0.01, P = 0.093)\). Furthermore, VDR/actin-mRNA did not correlate with PTH secretion in vitro.

**Discussion**

The aim of this study was to compare the magnitude of reduction in CaR expression with the level of impairment in Ca-regulated PTH release by human hyperplastic parathyroid glands measured in vitro. The results showed that for an equal amount of parathyroid tissue, a low CaR expression was associated with a high rate of PTH secretion, even when the Ca concentration in the medium was normal. Furthermore, glands with low CaR expression demonstrated a reduced PTH secretory response to both the inhibitory effect of high Ca and the stimulatory effect of low Ca. In addition, the setpoint of the PTH-Ca curve was high in glands with low CaR expression as compared with glands with relatively higher CaR expression. The study also showed that the larger the gland, the lower the CaRmRNA expression. Thus, large parathyroid glands secrete large amounts of PTH not only as a result of the increased gland size but also because individual cells seem to secrete more PTH.

Experiments were performed in parathyroid slices to maintain tissue architecture and preserve CaR expression (19–22). Because parathyroid tissue was cut into small pieces that were mixed thoroughly, the aliquots used for the experiments were representative of the entire gland. Depending on the amount of tissue available, studies of PTH secretion were repeated up to four times in the same gland. We observed that PTH secretion rates that were measured using different aliquots from the same gland were relatively uniform, suggesting that the values of PTH secretion obtained were indeed representative of the entire gland. The correlation observed between CaRmRNA and the CaR protein confirmed that CaRmRNA reflected the amount of CaR protein present in the parathyroid gland and suggested that in these glands, the transcription to protein was intact.

Although expression of the CaR tended to correlate with that of the VDR, the relationship was not statistically significant \((P = 0.09)\). This is not a rare finding, because other authors have observed that in secondary parathyroid hyperplasia, CaR expression did not correlate with VDR expression (by in situ hybridization) or even within the same parathyroid nodule (23). Thus, a possible interpretation of the current data is that although both VDR and CaR expression are decreased in advanced (nodular) parathyroid hyperplasia, the monoclonal origin of each nodule is different, so expression of genes may vary between glands. Other authors, however, have found that by immunohistochemical staining, CaR levels correlated with VDR levels in secondary parathyroid hyperplasia (24), although only low CaR, independent of VDR, was associated with high cell proliferation rate. We found that CaR but not VDR expression correlated inversely with parathyroid gland weight. As observed by other authors (6,9), our results showed that neither CaR nor VDR expression correlated with preoperative values of PTH and Ca. This lack of correlation may reflect that all of our patients had severe secondary hyperparathyroidism that required parathyroidectomy with serum concentrations of PTH and Ca limited to the high range. Furthermore, the biochemical severity of secondary hyperparathyroidism may be determined not only by CaR expression but also by total parathyroid gland weight, secretory output per cell, setpoint, and skeletal resistance to PTH.

We observed a wide range of CaR expression among the parathyroid glands evaluated in this study, which is in agreement with other reports (9,10,22,23). We did not have normal parathyroid tissue available for comparison; however, we assume that the CaR expression was decreased to the same degree as reported by others in the same type of hyperplastic parathyroid glands (9,10,22). In those studies, the parathyroid CaR content ranged from very low to normal levels. Therefore, it is likely that in this study, glands with the highest CaR had normal CaR expression. Inspection of the curvilinear relationship between PTH secretion and CaR/actin-mRNA (Figure 1) reveals that in glands from group I (CaR/actin-mRNA <4), PTH secretion was markedly increased. Similar observations can be made regarding the stimulation or suppression of PTH secretion by low and high Ca, respectively. PTH-Ca curves were performed in vitro to obtain setpoints; the data demonstrated that the setpoint was inversely correlated with the CaR expression. These findings suggest that only patients with advanced hyperparathyroidism and marked reductions in parathyroid CaR expression have overt abnormalities in the regulation of PTH secretion by Ca such as an increase in the setpoint (25).

Glands with low CaR expression had high baseline PTH secretion rates despite normal Ca concentration in the medium, increased setpoints, and failed to reduce PTH secretion after exposure to high extracellular Ca. We did not perform in vivo studies to evaluate the setpoint of PTH stimulation by Ca in the same patients included in this study.
However, all patients included in this study had severe hyperplasia, which has been associated with decreased sensitivity of parathyroid glands to Ca or increased setpoint of PTH stimulation by Ca (15,25). In primary hyperparathyroidism, other authors have shown an association between reduced parathyroid CaR expression and both increased setpoint (26) and increased PTH secretion (27).

The regulation of PTH secretion by Ca was analyzed in relation to baseline PTH secretion (Figures 4 and 5). Glands with high baseline PTH secretion rates have a poor secretory response to changes in extracellular Ca. According to our results, these glands usually have low CaR expression, and they are large; thus, the preservation of part of the smallest parathyroid gland when performing subtotal parathyroidectomy seems to be a reasonable strategy.

In conclusion, this study demonstrates that in hyperplastic parathyroid glands obtained from patients with ESRD, low parathyroid CaRmRNA expression is associated with high secretory output and deficient control of PTH secretion by extracellular Ca.

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