The influence of the progression of secondary hyperparathyroidism on the set point of the parathyroid hormone-calcium curve

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Abstract

The influence of secondary hyperparathyroidism (2 HPT) on the set point of the parathyroid hormone (PTH)-Ca^{2+} curve is controversial. *In vitro* experiments have shown an increase in the set point. However, clinical studies with hemodialysis patients have provided a variety of results (increases, decreases and no changes in the set point have been reported). The present study was designed to investigate the influence of the progression of 2 HPT on the set point of the PTH-Ca^{2+} curve. The PTH-Ca^{2+} curve and the expression of parathyroid calcium receptor (CaR mRNA) and vitamin D receptor (VDR mRNA) have been studied in normal rabbits (group I, $n=9$) and in nephrectomized rabbits (group II, $n=18$) at two stages after inducing 2 HPT: 2–3 weeks (group IIA) and 5–6 weeks (group IIB). In group I, the set point of the PTH-Ca^{2+} curve was $1{\cdot}63 \pm 0{\cdot}03$ mM. A progressive hypocalcemia was detected during the evolution of 2 HPT (groups IIA and IIB). Rabbits from group IIA had a significant ($P<0{\cdot}001$) decrease in the set point to values of $1{\cdot}45 \pm 0{\cdot}02$ mM. However, the set point increased significantly in group IIB ($P<0{\cdot}001$) to $1{\cdot}56 \pm 0{\cdot}03$ mM. CaR mRNA was similarly decreased in groups IIA ($39 \pm 12\%$) and IIB ($48 \pm 7\%$). No changes were detected in VDR mRNA. In conclusion, a reduction in the set point of the PTH-Ca^{2+} curve in response to decreased extracellular Ca^{2+} was detected in the early phases of 2 HPT. However, with the progression of 2 HPT the set point tended to increase even though extracellular Ca^{2+} was markedly decreased. The increase in the set point in the course of 2 HPT seems to be a complex process that cannot be fully explained by changes in parathyroid CaR mRNA or VDR mRNA.

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Introduction

Secondary hyperparathyroidism (2 HPT) is a common complication of end-stage renal disease. The decrease in functioning renal mass results in hypocalcemia, hyperphosphatemia and reduced calcitriol levels which stimulate parathyroid hormone (PTH) secretion and synthesis and promote parathyroid gland hyperplasia (Silver *et al.* 2002).

The relationship between PTH and blood ionized calcium (Ca^{2+}) is best represented by a sigmoidal curve: the PTH-Ca^{2+} curve. The set point of the PTH-Ca^{2+} curve, which is defined as the Ca^{2+} level at which PTH secretion equals 50% of maximal PTH, reflects the sensitivity of the parathyroid glands to changes in calcium (Felsenfeld & Llach 1993).

The influence of 2 HPT on the set point of the PTH-Ca^{2+} curve is controversial. *In vitro* experiments using parathyroid glands excised from azotemic patients with severe 2 HPT have shown an increase in the set point, which may represent an intrinsic abnormality of PTH secretion (Brown *et al.* 1982). However, clinical studies with hemodialysis patients have provided a variety of results. Ramirez *et al.* (1993) and Goodman *et al.* (1995) reported no changes in the set point of patients with 2 HPT. Malberti *et al.* (1993) and Goodman *et al.* (1998) did not find changes in the set point in patients with moderate 2 HPT but described an increase in the set point in patients with severe 2 HPT. By contrast, a decrease in the set point has been reported in uremic patients (Borrego *et al.* 1997, Cardinal *et al.* 1998).

On the other hand, several investigators have reported that the set point of the PTH-Ca^{2+} curve changes in concordance with variations in extracellular Ca^{2+} – i.e. decreases with hypocalcemia and increases with hypercalcemia (Pahl *et al.* 1996, Rodriguez *et al.* 1997, Hardy-Yverneau *et al.* 1998, Malberti *et al.* 1999).

Therefore, low extracellular Ca^{2+} seems to decrease the set point of the PTH-Ca^{2+} curve but persistently low Ca^{2+} levels are also known to generate parathyroid hyperplasia and 2 HPT which may increase the set point.

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The work reported here was designed to investigate the influence of the progression of 2 HPT on the set point of the PTH-Ca^{2+} curve.

Materials and Methods

Animals

White New Zealand rabbits of both sexes, aged 9–15 months and weighing 3·8 ± 0·1 kg, were used in the experiments. Rabbits were randomly assigned to any of the following groups.

Group I – control (n=9). These animals were used as a control, to obtain the PTH-Ca^{2+} curve in normal rabbits. They were fed *ad libitum* on a balanced diet containing Ca=1·2% and P=0·6% (normal diet). The hypercalcemic and hypocalcemic parts of the PTH-Ca^{2+} curve were obtained in the same animals with an interval of 1 week to avoid the phenomenom of hysteresis (Aguilera-Tejero *et al.* 1996, *Bas et al.* 2002, 2003).

Group II – 2 HPT (n=23). In these rabbits renal failure was induced by performing a five-sixth nephrectomy as previously described (Bas *et al.* 2004). Briefly, nephrectomy was completed in two stages: in the first stage, two-thirds of the cortical parenchyme of the left kidney was ablated and a week later a contralateral nephrectomy was performed. These rabbits were fed the normal diet for 2 weeks after performing the nephrectomy and then were switched to a pelleted synthetic diet containing Ca=0·6% and P=1·2% (low calcium–high phosphate diet (LCaHPD)). The reason for feeding the normal diet during the first 2 weeks is that many rabbits do not survive if they are fed an LCaHPD diet immediately after nephrectomy. After 2 weeks on the LCaHPD diet the hypercalcemic part of the PTH-Ca^{2+} curve was obtained. One week later the hypocalcemic part of the curve was obtained in the same rabbits. Thus, the first PTH-Ca^{2+} curve was obtained, in 18 rabbits, after 2–3 weeks on the LCaHPD (short-term hyperparathyroidism; group IIA). Five rabbits from group IIA were killed at 3 weeks to study their parathyroid glands. Rabbits were maintained on the same diet and the PTH-Ca^{2+} curve was studied again, in nine rabbits, at 5–6 weeks (medium-term hyperparathyroidism; group IIB).

At the end of the experiments rabbits were killed by an overdose of barbiturates. Parathyroid glands were removed from five rabbits of each group (control, group IIA and group IIB).

All animals received humane care in compliance with the 'Principles of Laboratory Animal Care' formulated by the National Society for Medical Research and the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Sciences and published by the National Institutes of Health. The experimental protocols of the present study were approved by the Ethics Commitee for Animal Research of the University of Cordoba.

PTH-Ca^{2+} curves

PTH-Ca^{2+} curves were obtained by i.v. infusion of disodium EDTA and $CaCl_2$. In all experimental groups, rabbits were anesthetized by a combination of ketamine (40 mg/kg) and midazolam (1 mg/kg). The marginal auricular vein and the central auricular artery were cannulated with 24 gauge catheters. The venous port was used for disodium EDTA and $CaCl_2$ infusion and the arterial side for blood sampling. The protocols for induction of hyper- and hypocalcemia were as follows. Hypercalcemia was achieved by i.v. infusion of $CaCl_2$ which was started at a rate of 0·175 mmol Ca/kg per h. $CaCl_2$ infusion was increased every 5 min up to a final rate of 1·75 mmol of Ca/kg per h at 45 min. Hypocalcemia was induced by an EDTA infusion which was initiated at a rate of 50 mg Na_2EDTA/kg per h. To achieve a linear decrease in Ca^{2+}, the rate of the EDTA infusion was progressively increased every 5 min, up to 190 mg/kg per h at the end of the experiment (45 min). When basal calcium was below normal, the duration of the hypocalcemic stimulus was adjusted to achieve a final $Ca^{2+}$of 1 mM.

Three blood samples were obtained from each animal as baseline; thereafter, blood samples were collected every 5 min until the end of the experiments. Samples were immediately centrifuged. Plasma was separated and Ca^{2+} and pH were measured using selective electrodes (Bayer Diagnostics, Barcelona, Spain) and then plasma was frozen at –70 °C. PTH was measured on plasma samples within 3 months of collection using an immunoradiometric assay which has previously been validated for quantitation of rabbit PTH (Allegro Intact PTH; Nichols, San Juan Capistrano, CA, USA) (Warren *et al.* 1989, Bas *et al.* 2002, 2003).

Individual PTH-Ca^{2+} curves were constructed by adjusting the PTH and Ca^{2+} values of every rabbit to a sigmoidal equation using the software SPSS 8·0 for Windows (SPSS Inc., Chicago, IL, USA). The PTH concentrations at standardized Ca^{2+} levels (from Ca^{2+}=2·1 mM to Ca^{2+}=1mM, with an interval of 0·05 mM) were extrapolated from these individual curves. Mean PTH values at standardized Ca^{2+} concentrations were used to obtain the PTH-Ca^{2+} curve for each group.

The following parameters were derived from the PTH-Ca^{2+} curves (Felsenfeld & Llach 1993).

(1) Basal PTH (PTHb) was the PTH concentration before initiating either hyper- or hypocalcemia.

(2) Maximal PTH (PTHmax) was the highest PTH level observed in response to hypocalcemia and additional reduction in plasma Ca^{2+} did not further increase PTH value.

(3) Minimal PTH (PTHmin) was the lowest PTH level during suppression by hypercalcemia and a further increase in plasma Ca^{2+} did not result in any additional increase in PTH.

(4) The ratio of basal to maximal PTH (PTHb/max) was the basal PTH divided by the maximal PTH and this fraction was multiplied by 100 to obtain a percentage.

(5) The basal plasma Ca^{2+} (Cab) was the plasma Ca^{2+} concentration before initiating either hyper- or hypocalcemia.

(6) The plasma Ca^{2+} at maximal PTH (Camax) was the plasma Ca^{2+} concentration at which the PTH level was first observed to be maximal.

(7) The plasma Ca^{2+} at minimal PTH (Camin) was the plasma Ca^{2+} concentration at which the PTH level was first observed to be minimal.

(8) The set point of Ca^{2+} was calculated in two different ways: (a) as the plasma Ca^{2+} concentration at which maximal PTH secretion was reduced by 50% (SP1) and (b) as the plasma Ca^{2+} concentration at which the difference between PTHmax and PTHmin was reduced by 50% (SP2).

Assessment of parathyroid gland hyperplasia

Glands were dissected free of adipose tissue and wet glandular weight was measured. The mean of five consecutive measurements was obtained from each gland. Parathyroid cell cycle was studied by flow cytometry as previously described (Canalejo *et al.* 2000). Briefly, small pieces of parathyroid tissue were viewed under an inverted microscope (×10) and were stripped apart using sharp Dumont forceps. This was followed by gently pipetting. To maintain high cell concentrations, these manipulations were performed in a small volume (30 μl) of phosphate-buffered saline (PBS). Dispersed cells were first treated with PBS containing 1% Triton X-100 at 37 °C, then with DNase-free RNase (10 μg/ml for 10 min) and finally with propidium iodide (20 μg/ml for 30 min) at 37 °C in the dark. Cells were immediately acquired by flow cytometer (FACScan; Becton-Dickinson, San Jose, CA, USA). LYSYS II software (Becton-Dickinson) was used for data acquisition and analysis. Cell debris and clumps were excluded from analysis by gating. The cell cycle was analyzed using the CELLFIT software (Becton-Dickinson). This method measures the percentage of cells in the different phases of the cell cycle: cells in G0/G1 phase are diploid cells, cells in phase S show an increase in the synthesis of DNA that precedes cell duplication and cells in G2+M have doubled the DNA content or are undergoing mitosis. The percentage of cells in phase S was used as a marker of cell proliferation.

Determination of calcium receptor (CaR) mRNA and vitamin D receptor (VDR) mRNA: RNA isolation and RT-PCR

Parathyroid tissue samples were placed in nuclease-free 1·5 ml microcentrifuge tubes for total RNA extraction. The tissue was ultrasonicated for 5 min at 4 °C and immersed in liquid nitrogen (−196 °C) to allow for complete cell rupture. Then, 400 μl of a lysis buffer from a commercial kit for tissue total RNA extraction (Mammalian Total RNA miniprep kit; Sigma, St Louis, MO, USA) was added to the samples. Total RNA was extracted using the Mammalian Total RNA miniprep kit. Extracted total RNA was dissolved in nuclease-free water (Promega, Madison, WI, USA) and heated for 10 min at 70 °C. The CaR and VDR vs β-actin were amplified with the c-Master RT-PCR kit (Eppendorff, Hamburg, Germany). The sequences of the primers were: CaR: sense (6-carboxyfluorescein (6 FAM)): 5′-ATTGAGGG GGAGCCCACCTGCTGCT-3′, anti-sense: 5′-AAAG AGGGTGAGTGCGATCCCAAAGG-3′ and VDR: sense (6 FAM): 5′-TGAAGGCTGCAAAGGCTTCA GGC-3′, anti-sense: 5′-GGATGAACTCCTTCATCA TGCCGATG-3′. Each sense primer was marked with 6 FAM fluorocrome. DNA amplifications were processed by a Genetic Analyzer Abi Prism 310 (Perkin Elmer, Foster City, CA, USA). Data were analyzed using specific software, Gene Scan version 3·1/1998 (Perkin Elmer). The basepair standard (size standard) was marked with a different type F fluorochrome (Tamra-350; Perkin Elmer) and run in parallel. The amount of CaR/β-actin mRNA and VDR/β-actin mRNA was expressed as percent of the control group.

Biochemical measurements

In addition to the measurements of Ca^{2+} and PTH, the following analytes were measured in baseline samples: inorganic phosphate, creatinine and calcitriol. Phosphate and creatinine were measured using standard spectrophotometric techniques (Sigma Diagnostics). Calcitriol was measured using a radioreceptor assay (Immunodiagnostic Systems, Bolton, Lancs, UK).

Statistics

For the intra- or intergroup comparison of three or more samples, repeated ANOVA was used. If the ANOVA showed statistical differences, a post hoc test, the Fisher HSD test, was used to determine differences. The Pearson test was used in the analysis of correlation. A P value$<0·05$ was considered significant. Results are expressed as the means ± S.E.M.

Results

Table 1 shows plasma concentrations of phosphate, creatinine and calcitriol in the three groups of rabbits. Phosphate was elevated in the 2 HPT groups. As expected, rabbits from group II (A and B) presented higher creatinine levels and lower calcitriol concentrations than the control group.

Figure 1 shows the PTH-Ca^{2+} curve in groups I, IIA and IIB. At baseline Ca^{2+}, 1·71 ± 0·05 mM, normal

Table 1 Plasma concentrations (means ± S.E.M.) of phosphate, creatinine and calcitriol in the three groups of rabbits: group I=control and group II=nephrectomized rabbits that had been fed a low Ca–high P diet for 2–3 weeks (IIA) and for 5–6 weeks (IIB)

	Phosphate (mmol/l)	**Creatinine** (μmol/l)	**Calcitriol** (pmol/l)
Group			
I (n=9)	1·06 ± 0·06	79·5 ± 8·8	125·7 ± 9·8
IIA (n=18)	3·01 ± 0·29[a]	397·8 ± 26·5[a]	30·9 ± 4·6[a]
IIB (n=9)	4·17 ± 0·45[a,b]	468·5 ± 53·1[a]	30·5 ± 5·0[a]

[a]P<0·05 when compared with group I, [b]P<0·05 when compared with group IIA.

rabbits (group I) had a PTH concentration of 26·9 ± 3·2 pg/ml. Induction of hypocalcemia resulted in an increase in PTH concentration that reached a maximum, 94·4 ± 5·5 pg/ml, at Ca^{2+} levels of 1·4 mM. At 2–3 weeks, nephrectomized rabbits (group IIA) had a lower baseline Ca^{2+}, 1·28 ± 0·03 mM, than group I (P<0·001). Basal (318 ± 22 pg/ml), maximal (366 ± 22 pg/ml) and minimal (48 ± 7 pg/ml) PTH concentrations in group IIA

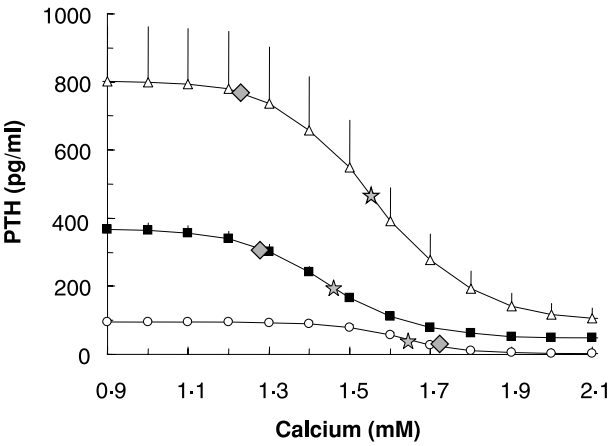

Figure 1 The PTH-Ca^{2+} curve in control rabbits (group I (○), n=9) and in nephrectomized rabbits after 2–3 weeks (group IIA (■), n=18) and 5–6 weeks (group IIB (△), n=9) of 2 HPT. Within each curve, baseline Ca^{2+} is marked by a diamond and the set point by a star. PTH (pmol/l)=PTH (pg/ml) × 0·105. Values are means ± S.E.M.

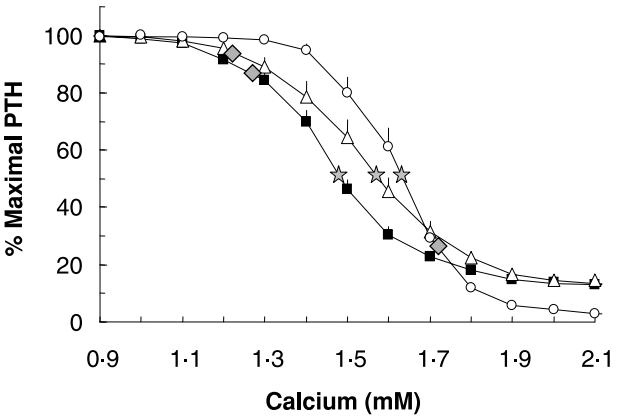

Figure 2 The PTH-Ca^{2+} curve with PTH concentrations expressed as a percentage of maximal PTH in control rabbits (group I (○), n=9) and in nephrectomized rabbits after 2–3 weeks (group IIA (■), n=18) and 5–6 weeks (group IIB (△), n=9) of 2 HPT. Within each curve, baseline Ca^{2+} is marked by a diamond and the set point by a star. PTH (pmol/l)=PTH (pg/ml) × 0·105. Values are means ± S.E.M.

were significantly higher than in group I (P<0·001). After 5–6 weeks, nephrectomized rabbits (group IIB) had a baseline Ca^{2+}, 1·22 ± 0·08 mM, that was lower than group I (P<0·001) but not significantly different from group IIA (1·28 ± 0·03 vs 1·22 ± 0·08 mM, P=0·37). In group IIB, PTHb (739 ± 155 pg/ml), PTHmax (801 ± 169 pg/ml) and PTHmin (102 ± 21 pg/ml) were significantly higher (P<0·05) than in the other groups.

The PTH-Ca^{2+} curves, with PTH values expressed as a percentage of PTHmax, are presented in Fig. 2. In normal rabbits (group I), the set point of the PTH-Ca^{2+} curve was 1·63 ± 0·03 mM. Rabbits from group IIA had a significant (P<0·001) decrease in the set point to values of 1·45 ± 0·02 mM. However, after 5–6 weeks of 2 HPT (group IIB), the set point increased significantly (P<0·001) to 1·56 ± 0·03 mM and was not different from the control group. Set point values were similar when calculated as 50% of PTHmax (SP1) or as 50% of PTHmax − PTHmin (SP2) (Table 2).

The relationship between the set point of the PTH-Ca^{2+} curve and baseline Ca^{2+} in groups IIA and IIB is

Table 2 Mean ± S.E.M. analytes and parameters derived from the PTH-Ca^{2+} curve in the three groups of rabbits: group I=control and group II=nephrectomized rabbits that had been fed a low Ca–high P diet for 2–3 weeks (IIA) and for 5–6 weeks (IIB)

	PTHb (pg/ml)	**PTHmax** (pg/ml)	**PTHmin** (pg/ml)	**PTHb/max** (%)	**Cab** (mM)	**Camax** (mM)	**Camin** (mM)	**SP1** (mM)	**SP2** (mM)
Group									
I (n=9)	26·9 ± 3·2	94·4 ± 5·5	3·2 ± 0·3	28 ± 3	1·71 ± 0·02	1·49 ± 0·03	1·97 ± 0·02	1·63 ± 0·02	1·62 ± 0·2
IIA (n=18)	318·1 ± 22·2[a]	366·1 ± 22·7[a]	48·1 ± 6·9[a]	87 ± 2[a]	1·28 ± 0·04[a]	1·26 ± 0·03[a]	1·90 ± 0·03[a]	1·45 ± 0·02[a]	1·42 ± 0·02[a]
IIB (n=9)	738·6 ± 155·8[a,b]	801·1 ± 169·4[a,b]	102·2 ± 22·2[a,b]	93 ± 2[a]	1·22 ± 0·08[a,b]	1·31 ± 0·04[a]	1·93 ± 0·02	1·56 ± 0·03	1·53 ± 0·03

[a]P<0·05 when compared with group I, [b]P<0·05 when compared with group IIA.
PTH (pmol/l)=PTH (pg/ml) × 0·105.

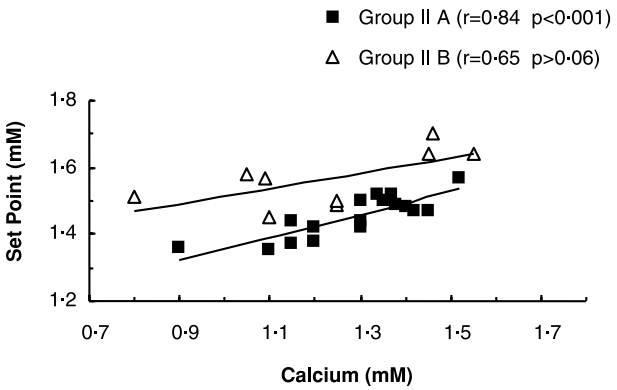

Figure 3 Correlation between baseline Ca^{2+} and the set point of the PTH-Ca^{2+} curve in nephrectomized rabbits after 2–3 weeks of 2 HPT (group IIA (■), $n=18$) and after 5–6 weeks of 2 HPT (group IIB (△), $n=9$).

presented in Fig. 3. After 2–3 weeks of 2 HPT (group IIA), a significant correlation between baseline Ca^{2+} and the set point was found ($r=0{\cdot}84$, $P<0{\cdot}001$). However, after 5–6 weeks of 2 HPT (group IIB), the correlation between baseline Ca^{2+} and the set point decreased and statistical significance was lost ($r=0{\cdot}65$, $P=0{\cdot}06$). Although the difference in the number of observations from each group may have influenced the r values, it is interesting to note that, in a broad range of extracellular Ca^{2+}, the set point was consistently higher in group IIB than in group IIA.

Parathyroid glands from normal rabbits weighed $6{\cdot}2 \pm 0{\cdot}3$ mg and had $0{\cdot}51 \pm 0{\cdot}01\%$ of cells in the S phase. A significant ($P<0{\cdot}05$) increase in glandular weight ($17{\cdot}2 \pm 2{\cdot}4$ mg) and percent of cells in the S phase ($3{\cdot}35 \pm 0{\cdot}8\%$) were identified after 5–6 weeks of 2 HPT (group IIB).

The CaR mRNA expression (CaR/β-actin mRNA) was markedly reduced, when compared with the control group ($100 \pm 19\%$), in both rabbits with short-term hyperparathyroidism (group IIA $= 39 \pm 12\%$, $P<0{\cdot}05$) and rabbits with medium-term hyperparathyroidism (group IIB $= 48 \pm 7\%$, $P<0{\cdot}05$). CaR mRNA values in groups IIA and IIB were not significantly different. A significant negative correlation ($P<0{\cdot}05$) was found between CaR mRNA expression and both glandular weight and maximal PTH secretion.

VDR mRNA/β-actin mRNA levels were slightly lower in both 2 HPT groups (group IIA $= 78{\cdot}2 \pm 13{\cdot}5\%$ and group IIB $= 85{\cdot}3 \pm 17{\cdot}2\%$) when compared with the control group $= 100 \pm 8{\cdot}2\%$. However, differences were not significant. In addition, VDR mRNA levels were not correlated with other indicators of hyperparathyroidism (glandular weight and maximal PTH secretion).

Discussion

The objective of the present study was to investigate the influence of the progression of 2 HPT on the set point of the PTH-Ca^{2+} curve. Our results showed a reduction in the set point which correlates with the decrease in extracellular Ca^{2+} 2–3 weeks after the induction of hyperparathyroidism. However, progression of parathyroid gland hyperplasia tended to increase the set point even though extracellular Ca^{2+} was markedly reduced.

The rabbit has been chosen, in the present study, as an animal model to investigate the effect of the progression of short and medium term 2 HPT on the set point of the PTH-Ca^{2+} curve. The rabbit model has the advantages of being an easily accessible laboratory animal whose body size allows the generation of detailed PTH-Ca^{2+} curves. Although normal rabbits on a standard diet have higher levels of extracellular Ca^{2+} than most mammals (around 1·7 mM), the PTH-Ca^{2+} relationship is comparable in rabbits, humans and other mammals (basal PTH, at $Ca^{2+}=1{\cdot}7$ mM, represents aproximately 25% of maximal PTH and both PTHmax and PTHmin are similar to the values obtained in humans). The rabbit model also has the advantage that extracellular Ca^{2+} levels are consistently and markedly decreased in the course of 2 HPT, thus facilitating study of the influence of hypocalcemia on the set point of the PTH-Ca^{2+} curve. In addition, rabbit PTH can be measured using the immunoradiometric assay for intact human PTH (Warren *et al.* 1989, Bas *et al.* 2002, 2003).

When obtaining the PTH-Ca^{2+} curves, the hypercalcemic part of the curve was always completed first to minimize the risk of death – uremic rabbits tolerate hypercalcemia better than hypocalcemia. To avoid any influence of the hypercalcemic stimulus on the subsequent hypocalcemia (hysteresis) a week was allowed before performing the hypocalcemic part of the curve (Aguilera-Tejero *et al.* 1996, Bas *et al.* 2002, 2003).

Nephrectomy plus the LCaHPD resulted in a progressive hypocalcemia and hyperphosphatemia. In addition, calcitriol levels were reduced in uremic rabbits. As a consequence, these rabbits developed parathyroid gland hyperplasia as demonstrated by an increase in glandular weight and an elevated cellular proliferation. The evolution of plasma PTH concentrations reflected progression of parathyroid gland hyperplasia. All PTH parameters (PTHb, PTHmax, PTHmin and the ratio PTHb/PTHmax) were more elevated at 5–6 weeks (group IIB) than at 2–3 weeks (group IIA).

At 2–3 weeks after the onset of hyperparathyroidism, rabbits from group IIA experienced a decrease in the set point of the PTH-Ca^{2+} curve which was highly correlated with the decrease in Cab. Thus, in these rabbits it seems that there was an adaptation of the set point to the ambient Ca^{2+}, even though they had already initiated parathyroid hyperplasia. These results are in agreeement with previous studies that have reported a decrease in the set point of the PTH-Ca^{2+} curve in uremic patients with decreased Ca^{2+} levels (Borrego *et al.* 1997). A decrease in the set point coincident with a reduction in extracellular Ca^{2+} has also

been reported in vitamin D-deficient dogs fed a low calcium diet (Cloutier *et al.* 1992); however, it is difficult to compare these data with the present study since in the vitamin D-deficient dog model hypocalcemia is a late event that appears well after 2 HPT has started.

Rabbits from group IIB, which had been developing 2 HPT for 5–6 weeks, were also markedly hypocalcemic. However, in these rabbits the set point increased when compared with group IIA, approaching the set point of normal rabbits. Thus, the correlation between Cab and the set point decreased at 5–6 weeks of 2 HPT. The increase in the set point found at 5–6 weeks, when compared with 2–3 weeks, is in agreement with previous reports that have identified a tendency to an increased set point of the PTH-Ca^{2+} curve in the course of primary (Brown *et al.* 1979) and secondary (Brown *et al.* 1982, Malberti *et al.* 1993, Goodman *et al.* 1998) hyperparathyroidism. However, in these studies the progression of hyperparathyroidism was accompanied by an increase in extracellular Ca^{2+} while in our study plasma Ca^{2+} remained low.

The increase in the set point in the course of 2 HPT, which reflects a decreased sensitivity of the parathyroid cells to Ca^{2+}, could be related to the reduction in CaR expression. A decrease in CaR has been described previously both in primary (Cetani *et al.* 2000) and secondary (Gogusev *et al.* 1997) hyperparathyroidism. Moreover, in patients with primary hyperparathyroidism the reduction in CaR has been reported to correlate with the increase in the set point (Cetani *et al.* 2000). Our results confirm a decrease in CaR in the course of 2 HPT. However, as previously reported (Ritter *et al.* 2001) the reduction in CaR expression occurs early in the course of 2 HPT (before the set point begins to increase); thus, other factors related to parathyroid hyperplasia may also influence the increase in the set point detected with the progression of 2 HPT.

A decrease in VDR, which reduces the ability of calcitriol to inhibit parathyroid gland proliferation, is a well-documented etiopathogenic factor in both primary and secondary hyperparathyroidism (Carling *et al.* 2000, Lewin *et al.* 2002). The absence of changes in VDR expression in our study, which has also been reported in other models of experimental uremia (Szabo *et al.* 1996) and nutritional hyperparathyroidism (Hernandez *et al.* 1996), may be explained by the heterogenous distribution of VDR in the parathyroid glands (Fukuda *et al.* 1993, Valimaki *et al.* 2001). VDR mRNA has lower density in glands with chronic hyperparathyroidism showing nodular hyperplasia (Fukuda *et al.* 1993). However, in rabbits with short/medium term hyperparathyroidism, nodularity is not a significant feature (Bas *et al.* 2004). Thus the VDR mRNA results must be interpreted in the specific context of our study. Moreover, since in experimental models of uremia it is difficult to achieve the chronic hyperparathyroidism (nodular parathyroid hyperplasia) that is often found in patients receiving hemodialysis, care should be taken when extrapolating the results to humans with renal impairment.

In conclusion, a reduction in the set point of the PTH-Ca^{2+} curve has been detected in the early phases of 2 HPT. However, with the progression of parathyroid gland hyperplasia the set point tends to increase even though extracellular Ca^{2+} is still markedly reduced. The increase in the set point in the course of 2 HPT seems to be a complex process that cannot be fully explained by changes in parathyroid CaR mRNA or VDR mRNA.

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