EXPERIMENTAL STUDY

The influence of acute and chronic hypercalcemia on the parathyroid hormone response to hypocalcemia in rabbits

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Abstract

Objective: To investigate the influence of acute and chronic hypercalcemia on the parathyroid hormone (PTH) response to hypocalcemia.

Design: The PTH response to hypocalcemia has been evaluated in three groups of rabbits: Group I, normal rabbits, Group II, normal rabbits subjected to an acute hypercalcemic clamp (induced by CaCl_2 infusion) and Group III, rabbits with chronic hypercalcemia (due to surgical reduction of renal mass).

Results: In Group I (baseline $\text{Ca}^{2+} = 1.69 \pm 0.02$ mM), hypocalcemia resulted in stimulation of PTH secretion which reached a maximum (PTHmax) of 91.7 ± 6.4 pg/ml. In rabbits from Group II, which also had normal baseline Ca^{2+} (1.70 ± 0.02 mM), plasma Ca^{2+} was maintained at an elevated level for 2 h, at around 2.05 mM. The PTH response to hypocalcemia in Group II was attenuated and the PTHmax in these rabbits was 45.6 ± 7.4 pg/ml. In rabbits from Group III, baseline Ca^{2+} was elevated (2.06 ± 0.06 mM) for 1 month. The PTH response to hypocalcemia in Group III was essentially the same as in Group I and PTHmax reached levels of 94.8 ± 9.9 pg/ml.

Conclusions: A difference in PTH response to hypocalcemia has been found in rabbits after exposure to either acute or chronic hypercalcemia. After acute hypercalcemia, an attenuated PTH response to hypocalcemia has been identified. Chronic hypercalcemia, however, did not influence the PTH response to hypocalcemia.

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Introduction

The relationship between parathyroid hormone (PTH) and blood ionized calcium (Ca^{2+}) is best represented by a sigmoidal curve: the PTH– Ca^{2+} curve. The parathyroid glands have evolved to allow a wide range of response to hypocalcemia and thus basal PTH secretion is uniquely positioned at approximately 25% of the maximal PTH in normal humans and in most mammals (1, 2).

In a previous study, we reported a reduced PTH response to hypocalcemia after a short period of hypercalcemia. In these experiments, dogs were subjected to a hypercalcemic clamp (1.65 mM) for 2 h and then their parathyroid glands were stimulated by reducing blood Ca^{2+} . When compared with dogs in which hypocalcemia was initiated from basal Ca^{2+} (1.25 mM), the PTH response to hypocalcemia was less for the same Ca^{2+} when the reduction in Ca^{2+} was initiated from hypercalcemia than from a normal plasma calcium concentration (3).

This study raised the question of whether the parathyroid glands would also have a reduced response to hypocalcemia when exposed to chronic hypercalcemia. It is likely that some of the changes in PTH metabolism that have been described when parathyroid glands are exposed to acute hypercalcemia (4, 5) will also be present in chronic hypercalcemia (6). Moreover, chronic hypercalcemia has been reported to influence PTH gene transcription and decrease PTH mRNA levels (7, 8). Thus, theoretically, chronic hypercalcemia should also decrease the PTH response to hypocalcemia. However, it is also possible that over time the parathyroid glands become adapted to high levels of extracellular Ca^{2+} .

To answer this question an animal model in which chronic hypercalcemia could be maintained was needed. Rabbits are known to have higher levels of extracellular Ca^{2+} than most mammals (around 1.7 mM). However, Butters *et al.* (9) have demonstrated that the intrinsic functional properties of the rabbit calcium-sensing receptor (CaR) are indistinguishable

from those observed in the human CaR. It seems that, in rabbits, the set-point for control of parathyroid function by Ca^{2+} is positioned to defend a Ca^{2+} concentration that would be high in most species (10). It is also documented that, in rabbits, chronic renal failure (CRF) results in a further increase in extracellular Ca^{2+} when dietary calcium is unrestricted (11). Thus, the rabbit with CRF would be an appropriate model in which to study the effect of chronic hypercalcemia on the PTH response to hypocalcemia.

The objective of the present study was to investigate the PTH response to hypocalcemia in normal rabbits and in rabbits in which hypercalcemia was induced either acutely (hypercalcemic clamp) or chronically (CRF).

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. They were housed individually, had permanent access to water and were fed *ad libitum* a commercial diet containing $\text{Ca} = 1.2\%$ and $\text{P} = 0.6\%$. They were randomly assigned to one of three groups. Group I ($n = 10$): these animals were used as a control to obtain the PTH– Ca^{2+} curve in normocalcemic rabbits (plasma $\text{Ca}^{2+} = 1.7$ mM). The PTH– Ca^{2+} curve was also obtained in an additional group of normal rabbits, Group IB ($n = 5$), in which the rate of calcium reduction was modified to achieve lower levels of Ca^{2+} at the end of the experiments. Group II ($n = 9$): in these rabbits, an acute (2 h) hypercalcemic clamp was performed and afterwards the PTH response to hypocalcemia was evaluated. Plasma Ca^{2+} was increased from its baseline value (1.7 mM) to 2.05 mM during the first 30 min and then was maintained at an elevated level for an additional period of 90 min. Group III ($n = 8$): in this group, CRF was induced by performing a 5/6 nephrectomy, as a consequence of which the rabbits developed chronic hypercalcemia. Nephrectomy was completed in two stages: in the first stage 2/3 of the cortical parenchyma of the left kidney was ablated, a week later a contralateral nephrectomy was performed. Hypocalcemia was induced after the rabbits had been in CRF and hypercalcemic (2.05 mM) for 4 weeks.

PTH– Ca^{2+} curves

PTH– Ca^{2+} curves were obtained by i.v. infusion of disodium EDTA. In all experimental groups, rabbits were anesthetized by a combination of ketamine (40 mg/kg; Ketolar, Parke-Davis SL, Barcelona, Spain) and midazolam (1 mg/kg; Midazolam Rovi, Rovi SA, Madrid, Spain). The marginal auricular vein and the central auricular artery were cannulated with 24 gauge catheters. The venous port was used for EDTA

infusion and the arterial side for blood sampling. The protocols for induction of hypocalcemia were as follows. Group I: EDTA infusion was initiated at a rate of 50 mg/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 (30 min). In Group IB, the initial rate of EDTA was also 50 mg/kg per h and the rate of infusion was increased more rapidly so that the final rate was 400 mg/kg per h. Group II: in this group, a hypercalcemic clamp was performed before inducing hypocalcemia. Hypercalcemia was achieved by i.v. infusion of CaCl_2 at a mean rate of 1.5 mEq/kg per h for 30 min and was then maintained for 90 additional min by infusing CaCl_2 at a rate of 1.2 mEq/kg per h. After 120 min with hypercalcemia, hypocalcemia was induced, first by reducing the dose of CaCl_2 over 15 min and then by infusing EDTA at an initial rate of 25 mg/kg per h which was progressively increased to 240 mg/kg per h at the end of the experiment (60 min). Group III: basal calcium (2.05 mM) was reduced by an EDTA infusion that was initiated at a rate of 50 mg/kg per h and increased every 5 min up to a final rate of 450 mg/kg per h at the end of the experiment (60 min).

Three blood samples were obtained from each animal as baseline; subsequently, blood samples were collected every 5 min throughout the experiments. Samples were immediately centrifuged. Plasma was separated and Ca^{2+} and pH were measured using selective electrodes (Ciba-Corning Diagnostics, Madrid, Spain); plasma was then frozen at -70°C . PTH was measured on plasma samples within 3 months of collection using an immunoradiometric assay which has been previously validated for quantitation of rabbit PTH (Allegro Intact PTH; Nichols, San Juan Capistrano, CA, USA) (10).

Plasma concentrations of creatinine, calcitriol (CTR) and phosphate were also measured in baseline samples. Creatinine and phosphate were quantified by spectrophotometric methods (Sigma Diagnostics, St Louis, MO, USA). CTR was measured using a radioreceptor assay (Immunodiagnostic Systems, Bolton, UK).

Individual PTH– Ca^{2+} curves were constructed by adjusting the PTH and Ca^{2+} values of every rabbit to a sigmoidal equation. PTH concentrations at standardized Ca^{2+} levels (from $\text{Ca}^{2+} = 1$ mM to $\text{Ca}^{2+} = 2.05$ mM, 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.

Statistics

For the intra- or intergroup comparison of three or more samples, repeated analysis of variance (ANOVA) was used. If the ANOVA showed a statistical

difference, a post hoc test, the Fisher least significance difference test, was used to determine differences. A P value <0.05 was considered significant. Results are expressed as the means $\pm$ S.E.

Results

Table 1 shows parameters related with renal function in normal rabbits (Groups I and II) and rabbits with CRF (Group III). Plasma creatinine concentration was significantly higher in rabbits with CRF than in normal rabbits (3.8 ± 0.6 vs 0.9 ± 0.1 mg/dl, $P < 0.001$). Rabbits with CRF also had lower values of CTR (11.6 ± 1.3 vs 52.4 ± 4.1 pg/ml, $P < 0.001$) and phosphate (2.3 ± 0.2 vs 3.3 ± 0.2 mg/dl, $P = 0.011$). No differences in plasma pH were detected between normal and uremic rabbits (7.421 ± 0.007 vs 7.423 ± 0.012 , not significant (NS)).

The time-course for plasma Ca^{2+} and PTH in Groups I, II and III is presented in Fig. 1. In Group I, EDTA infusion caused a progressive decline in plasma Ca^{2+} from its baseline concentration of 1.69 ± 0.02 mM to 1.34 ± 0.04 mM at the end of the experiment (Fig. 1A). Hypocalcemia resulted in a steady increase in PTH secretion: plasma PTH levels rose from a basal value of 31.5 ± 6.1 pg/ml to a maximum (PTHmax) of 91.7 ± 6.4 pg/ml (Fig. 1B).

Rabbits from Group II had a basal plasma Ca^{2+} similar to Group I (1.70 ± 0.02 vs 1.69 ± 0.02 mM, NS). In these rabbits, CaCl_2 infusion increased the plasma Ca^{2+} over 30 min to a value of 2.11 ± 0.03 pg/ml; during the following 90 min, the plasma Ca^{2+} was clamped at levels around 2.05 mM. EDTA was then infused and plasma Ca^{2+} was decreased from 2.04 ± 0.03 to 0.95 ± 0.03 mM in a 60-min period (Fig. 1A). Basal PTH concentration was not different in Groups I and II (31.5 ± 6.1 vs 25.2 ± 6.1 , NS). During the hypercalcemic clamp, PTH concentration was significantly reduced to levels around 6 pg/ml. The subsequent hypocalcemia produced a moderate increase in PTH concentration which rose from 7.5 ± 0.1 pg/ml to only 45.6 ± 7.4 pg/ml (Fig. 1B).

Group III rabbits had a basal Ca^{2+} of 2.06 ± 0.06 mM; this Ca^{2+} level was significantly higher than the basal Ca^{2+} in Groups I and II (1.69 ± 0.02 and

1.70 ± 0.02 mM, $P < 0.001$) and similar to the Ca^{2+} concentration at the end of the hypercalcemic clamp in Group II (2.04 ± 0.03 mM, $P = 0.602$). During EDTA infusion, plasma Ca^{2+} was reduced over 60 min to reach a final concentration of 0.95 ± 0.04 mM (Fig. 1A). Baseline PTH in Group III rabbits (2.2 ± 0.3 pg/ml) was significantly lower than in Groups I (31.5 ± 6.1 pg/ml, $P = 0.004$) and II (25.2 ± 6.1 pg/ml, $P = 0.04$). During hypocalcemia, the PTH levels increased steadily up to 94.8 ± 9.9 pg/ml (Fig. 1B).

When studying the PTH- Ca^{2+} curve obtained in rabbits from Group I, at the standardized Ca^{2+} level that was closest to basal calcium (1.70 mM), the PTH concentration was 26.9 ± 4.2 pg/ml. Hypocalcemia caused an increase in PTH concentration up to a maximum of 91.7 ± 6.4 pg/ml which was achieved with a Ca^{2+} concentration of 1.35 mM. In Group II, in which the hypocalcemic stimulus was initiated from hypercalcemia, the PTH concentration for a standardized Ca^{2+} level of 2.05 mM was 4.1 ± 0.5 pg/ml. During hypocalcemia, PTH secretion was stimulated; however, at baseline Ca^{2+} (1.7 mM), PTH concentration was slightly lower than in Group I (17.5 ± 1.3 vs 26.9 ± 4.2 , $P = 0.19$) and further reductions in Ca^{2+} resulted in only a moderate increase in PTH that reached a maximum around 40 pg/ml at a Ca^{2+} level of 1.25 mM. A further decrease in plasma Ca^{2+} did not elicit an increase in PTH secretion. Moreover, within the Ca^{2+} concentration range of 1.65–1.0 mM, the PTH concentration was significantly lower in Group II than in Group I (Fig. 1C).

In Group III, the PTH- Ca^{2+} curve was initiated from the baseline hypercalcemic values. PTH concentration increased from a level below 2 pg/ml, at $\text{Ca}^{2+} = 2.05$ mM, to 24.6 ± 4.5 pg/ml, at $\text{Ca}^{2+} = 1.7$ mM. There were no differences in the PTH concentration at this Ca^{2+} level between Groups I and III (25.2 ± 4.2 vs 24.6 ± 4.5 pg/ml, NS). The PTH- Ca^{2+} curve at Ca^{2+} concentrations below 1.7 mM was very similar in Groups I and III, and no significant differences were detected between these two groups at any Ca^{2+} level (Fig. 1C).

A summary of the changes in Ca^{2+} and PTH in the three experimental Groups (I, II and III) is shown in Table 2.

Table 1 Biochemical parameters (means $\pm$ S.E.) in normal rabbits (Groups I and II) and rabbits with CRF (Group III).

	Creatinine (mg/dl)	CTR (pg/ml)	Phosphate (mg/dl)	pH
Normal rabbits ($n = 19$)	0.9 ± 0.1	52.4 ± 4.1	3.3 ± 0.2	7.421 ± 0.007
CRF rabbits ($n = 8$)	$3.8 \pm 0.6^*$	$11.6 \pm 1.3^*$	$2.3 \pm 0.2^*$	7.423 ± 0.012

CRF, chronic renal failure; CTR, calcitriol.

* $P < 0.05$ vs normal rabbits.

Figure 1 The time-course of Ca^{2+} (A) and PTH (B) and the PTH- Ca^{2+} curve (C) in Groups I, II and III. Values are the means $\pm$ S.E. (when the standard error bar is not present, it was too small to draw). Normocalcemic rabbits (Group I (●)), rabbits with acute hypercalcemic clamp (Group II (○)) and chronic hypercalcemic rabbits (Group III (□)). * $P < 0.05$, significant differences in PTH concentration, for the same Ca^{2+} level, with respect to Group I. All the PTH values corresponding to Ca^{2+} concentrations below 1.35 mM of Groups II and III have been compared with the maximum PTH value in Group I.

A comparative study of the hypercalcemic part of the PTH- Ca^{2+} curve in Groups II and III is presented in Fig. 2. At a standardized $\text{Ca}^{2+} = 2.05$ mM, the PTH concentration was significantly lower in Group III than

Figure 2 The hypercalcemic part of the PTH- Ca^{2+} curve in Groups II and III. Values are the mean $\pm$ S.E. Rabbits with acute hypercalcemic clamp (Group II (○)) and chronic hypercalcemic rabbits (Group III (□)). * $P < 0.05$, significant differences between groups.

in Group II (1.3 ± 0.3 vs 5.1 ± 0.6 pg/ml, $P < 0.001$). PTH concentrations remained significantly lower in Group III than in Group II at Ca^{2+} levels ranging from 2.05 to 1.85 mM. The PTH levels became similar in both groups at $\text{Ca}^{2+} = 1.75$ mM and, at the standardized Ca^{2+} level that is closest to the basal Ca^{2+} of normal rabbits (1.70 mM), the PTH concentration was slightly higher in Group III than in Group II.

A comparison of the time-course of Ca^{2+} in Groups I and III (Fig. 1) revealed that the final Ca^{2+} concentration was higher in Group I than in Group III. To exclude the possibility that this difference might have influenced the results, an additional control group (Group IB) was studied. Figure 3 shows the time-course of Ca^{2+} and PTH in Groups IB and III. In Group IB, the reduction of Ca^{2+} , from 1.64 to 0.85 mM, was achieved in 30 min. Thus, the rate of Ca^{2+} reduction was similar in Groups IB and III (Fig. 3A). Baseline Ca^{2+} concentration was slightly lower in Group IB than in Group I (1.64 ± 0.03 vs 1.69 ± 0.02 mM) and this also resulted in higher basal PTH levels (49.3 ± 12.8 vs 31.5 ± 6.1 pg/ml); however, these differences were not statistically significant. The changes in PTH in Group IB were practically identical to the changes in Group III, and there was no difference in the maximal PTH concentration observed in Groups IB and III (Fig. 3B). Moreover, the PTH- Ca^{2+} curves were similar in Groups IB and III, and no significant differences in PTH concentration were found at any Ca^{2+} level (Fig. 3C).

Discussion

The objective of the present study was to investigate whether the PTH response to hypocalcemia was

Table 2 Changes in Ca^{2+} and PTH in the three groups of rabbits (Group I = normal rabbits, Group II = normal rabbits subjected to a hypercalcemic clamp, Group III = rabbits with chronic hypercalcemia secondary to CRF). Values are means $\pm$ S.E..

	$\text{Ca}^{2+}(\text{b})$ (mM)	PTH(b) (pg/ml)	$\text{Ca}^{2+}(\text{min})$ (mM)	PTHmax (pg/ml)
Group I ($n = 10$)	1.69 ± 0.02	31.5 ± 6.1	1.34 ± 0.04	$91.7 \pm 6.4^{\text{b}}$
Group II ($n = 8$)	$2.04 \pm 0.03^{\text{a}}$	$7.5 \pm 0.1^{\text{a}}$	$0.95 \pm 0.03^{\text{a}}$	$45.6 \pm 7.4^{\text{a}}$
Group III ($n = 9$)	$2.06 \pm 0.06^{\text{a}}$	$2.2 \pm 0.3^{\text{a,b}}$	$0.95 \pm 0.04^{\text{a}}$	$94.8 \pm 9.9^{\text{b}}$

$\text{Ca}^{2+}(\text{b})$ = plasma calcium concentration at the beginning of the hypocalcemic stimulus. PTH(b) = plasma PTH concentration at the beginning of the hypocalcemic stimulus. $\text{Ca}^{2+}(\text{min})$ = plasma calcium concentration that elicited maximal stimulation of PTH secretion. PTHmax = maximum value of PTH obtained during the hypocalcemic stimulation of the parathyroid glands.

^a $P < 0.05$ vs Group I. ^b $P < 0.05$ vs Group II.

attenuated after the parathyroid glands had been exposed to chronic (1 month) hypercalcemia. Our results show that in contrast to acute hypercalcemia, which causes an attenuated PTH response to hypocalcemia, chronic hypercalcemia does not influence the PTH response to hypocalcemia.

In this study, the rabbit with CRF has been chosen as an animal model for chronic hypercalcemia. This model is convenient because hypercalcemia can be achieved without providing exogenous PTH or CTR supplementation. In normal rabbits on a standard diet, the plasma Ca^{2+} concentration is around 1.7 mM. This value, although relatively high as compared with most species, is normal for rabbits as reflected by the fact that PTH secretion in these animals, far from being inhibited, is approximately 25% of PTHmax. Moreover, the PTH response obtained in normal rabbits after an acute hypercalcemic clamp is similar to that which has been previously observed in dogs (3). Thus, the higher Ca^{2+} values in normal rabbits should not interfere with the objective of this work.

The mechanisms responsible for chronic hypercalcemia in rabbits with CRF are not clear. It is interesting to note that the horse, another mammal with a relatively high extracellular calcium, also develops hypercalcemia during CRF (12). Moreover, both species show a tendency to decreased plasma phosphate levels in conjunction with hypercalcemia (11, 12). These biochemical changes seem to be related to the fact that rabbits and horses have a higher net intestinal absorption of calcium than most mammals, and to maintain calcium homeostasis they rely, to a large extent, on renal excretion of calcium (fractional excretion of calcium can reach levels of 45%, as compared with values of 2% in other species). Thus it is likely that during CRF, if calcium intake is unrestricted, these animals fail to excrete the relatively high load of calcium absorbed in the intestine and develop hypercalcemia (11, 12).

In normal rabbits (Group I), baseline Ca^{2+} values and the PTH– Ca^{2+} curve were within the range reported in the literature (10, 13, 14). It is interesting to note that although plasma Ca^{2+} concentration in rabbits is higher than in most mammals, PTH values are com-

parable with those which have been reported in man, carnivores and rodents (1, 15, 16). This is not a unique feature of rabbits since in the horse, another mammal with high basal Ca^{2+} levels, PTH values have also been reported to be within the normal range of normocalcemic animals (17).

The results obtained during the hypercalcemic clamp (Group II) were also similar to those previously reported using the same protocol in dogs (3). However, as compared with the results obtained by Sánchez *et al.* (3) there are two points that should be noted. (a) The PTH response to hypocalcemia is even more attenuated after the hypercalcemic clamp in rabbits (50% of PTHmax) than in dogs (60% of PTHmax) and (b) in the study by Sánchez *et al.* (3) PTH secretion never reached a plateau, although the final Ca^{2+} was 0.8 mM ($\Delta \text{Ca}^{2+} = 0.8$ mM). Thus, in that study, there was a possibility that further reductions in Ca^{2+} may have resulted in a progressive increase in PTH to reach PTHmax. In our rabbits, however, PTH reached a maximum at $\text{Ca}^{2+} = 1.25$ mM and then remained stable at around 40 pg/ml, despite the fact that Ca^{2+} was decreased to 1.0 mM. In some rabbits, Ca^{2+} was lowered further, to 0.8 mM, and PTH continued at the same plateau (data not shown).

The reasons for the reduced PTH response to hypocalcemia after a short period of hypercalcemia are not clear. High levels of extracellular Ca^{2+} have been shown to promote intracellular PTH degradation both *in vivo* (5) and *in vitro* (4), thus an increase in intracellular PTH metabolism could be a major factor in the reduced PTH response to hypocalcemia found in Group II. Likewise, an elevation in extracellular Ca^{2+} concentration has also been shown to inhibit PTH biosynthesis by decreasing levels of PTH mRNA (7, 8). However, the decrease in PTH mRNA begins after 16 h of exposure to high Ca^{2+} (7); therefore changes in PTH synthesis are probably not relevant in our acute (2 h) experiments. It has also been speculated that secretory products could modify the PTH response to changes in plasma Ca^{2+} . In this context, it has been shown that, during hypercalcemia, secretion of intact PTH(1–84) is suppressed while secretion of carboxy-terminal PTH is less suppressed (18–20). In addition

Figure 3 The time-course of Ca^{2+} (A) and PTH (B) and the PTH- Ca^{2+} curve (C) in Groups IB and III are shown. Values are the means $\pm$ S.E. (when the standard error bar is not present, it was too small to draw). Normocalcemic rabbits (Group IB (■)) and chronic hypercalcemic rabbits (Group III (□)).

to the secretion of intact PTH and its fragments, several secretory products of the parathyroid glands like chromogranin A and its metabolites (21–23) and endothelin 1 (24) could play a role in the inhibition of PTH secretion after acute hypercalcemia. Finally, changes in intracellular sensing mechanisms triggered by the action of calcium on the CaR could also explain this phenomenon – 90 min of sustained hypercalcemia may alter

intracellular signaling (concentration of intracellular mediators, such as arachidonic acid) (25) resulting in a blunted secretory response to subsequent hypocalcemia.

This study was designed to test the hypothesis that chronic hypercalcemia would result in a decrease in the PTH response to hypocalcemia, similar to that previously described after acute hypercalcemia (3). However, the results obtained in Group III rabbits proved this hypothesis not to be true.

Since PTHmax did not decrease after 1 month with hypercalcemia, the size of the parathyroid glands was presumably unchanged during this period of time. However, basal PTH concentrations were very low in Group III rabbits. Thus, it is likely that the percentage of secretory cells was reduced during chronic hypercalcemia. This hypothesis, i.e. an increase in the percentage of cells that are capable of secretion but do not release PTH at baseline Ca^{2+} , would be in agreement with the heterogeneous response to changes in extracellular Ca^{2+} by individual parathyroid cells described by Sun *et al.* (26).

It is also interesting to note that when comparing PTH secretion in hypercalcemia (between Ca^{2+} 2.05 and 1.70 mM) in Groups II and III (Fig. 2), PTH levels are significantly higher in Group II (acute hypercalcemia) than in Group III (chronic hypercalcemia). This fact could be explained by the hysteresis of the PTH- Ca^{2+} curve. With respect to the PTH- Ca^{2+} relationship, the term hysteresis indicates that for the same plasma Ca^{2+} , the plasma PTH is lower during the acute induction of hypercalcemia than the acute recovery from hypercalcemia (27). Thus, in Group II rabbits, which have normal baseline Ca^{2+} (1.70 mM) and are recovering from an acute hypercalcemia, the hysteresis of the PTH- Ca^{2+} curve would increase PTH levels. This phenomenon is not observed in Group III rabbits which had been hypercalcemic (baseline Ca^{2+} = 2.06 ± 0.06 mM) for 1 month.

When this study was designed, a reduction in Ca^{2+} concentration (ΔCa^{2+}) of 0.4 mM (from 1.7 to 1.3 mM) over a period of 30 min was considered appropriate to obtain the PTH- Ca^{2+} curve in normal rabbits (Group I). This degree of hypocalcemia is known to maximally stimulate PTH secretion in all the species in which PTH- Ca^{2+} curves have been reported (1, 2, 3, 17) including rabbits (10). In Groups II and III, in which a reduced PTH response to hypocalcemia was anticipated, Ca^{2+} levels were further reduced, reaching a final Ca^{2+} concentration below 1.0 mM. Although the results obtained in Group I indicate that the maximum of PTH secretion had been attained (no significant changes in PTH concentration were detected in the last two samples even though there was a significant reduction in Ca^{2+}), it was decided to include a second control group of normal rabbits (Group IB) in which Ca^{2+} concentration was reduced to the same level as

that in Groups II and III. The study of this additional group was meant to ensure that the PTH response to hypocalcemia was not significantly different in normal rabbits and in rabbits subjected to chronic hypercalcemia. As can be appreciated in Fig. 3, the reduction in plasma Ca^{2+} below 1.0 mM did not increase the PTH response in normal rabbits. The PTH response to hypocalcemia was practically identical in Groups IB and III, thus confirming the results obtained in Group I and providing support for the hypothesis that chronic hypercalcemia does not modify the PTH response to hypocalcemia.

The difference between the parathyroid response to hypocalcemia after acute versus chronic hypercalcemia could be explained by the disappearance over time of any kind of regulation that would serve to explicate the reduced response to hypocalcemia after acute hypercalcemia. The signaling system coupled to the CaR may be adapted to chronic hypercalcemia; thus, despite a high ambient calcium which inhibits PTH secretion, the secretory response to hypocalcemia is normal. It is also possible that the changes induced by acute hypercalcemia persist during chronic hypercalcemia but may be compensated by the effect of the decreased CTR levels found in rabbits with CRF. A decrease in CTR is known to stimulate PTH synthesis and secretion (28–30) and thus could counteract the inhibitory effect of hypercalcemia. If this were the case, the influence of decreased CTR should be evident at any calcium level. While a decrease in CTR could serve to explain the normal PTH response to hypocalcemia in chronic hypercalcemic rabbits, it would be reasonable to expect that these rabbits also had increased PTH levels during hypercalcemia. However, as shown in Fig. 2, the PTH concentration at high Ca^{2+} levels (1.7–2.05 mM) in chronic hypercalcemic rabbits with decreased CTR is inferior to the PTH concentration at the same Ca^{2+} levels in acute hypercalcemic rabbits with normal CTR.

It is also interesting to note that even though the PTH response to calcium (PTH– Ca^{2+} curve) in chronic hypercalcemic rabbits is not different from that in normocalcemic rabbits, the parathyroid glands do not sense the chronic high Ca^{2+} as normal Ca^{2+} . This contention is supported by two facts. (a) At high Ca^{2+} levels PTH secretion is inhibited, actually it is maximally inhibited and (b) the parathyroid glands do not try to 'defend' this high Ca^{2+} level. If the glands were sensing the high Ca^{2+} as normal, they would respond with maximal stimulation to a significant reduction in Ca^{2+} . However, when challenged with a decrease in Ca^{2+} that would produce maximal secretion in normocalcemic rabbits ($\Delta \text{Ca}^{2+} = 0.35 \text{ mM}$), PTH is not maximally stimulated but rather reaches the same concentration as in normocalcemic rabbits with $\text{Ca}^{2+} = 1.70$. These data indicate that in rabbits subjected to chronic hypercalcemia, PTH concentration is determined mainly by the actual Ca^{2+} level.

This study also provides information on the changes in mineral metabolism during CRF in rabbits. It has long been known that rabbits with CRF experience an increase in Ca^{2+} ; however, to our knowledge, the PTH response in rabbits with CRF had not been reported. Our results indicate that rabbits with CRF have suppressed basal PTH levels and did not develop secondary hyperparathyroidism over the time-course of this study. Hyperparathyroidism secondary to renal failure is caused by a decrease in CTR and Ca^{2+} and an increase in phosphate. In rabbits with CRF, although CTR is decreased, Ca^{2+} is increased and phosphate is decreased and the net result is a decrease in PTH concentration. These results emphasize the role of Ca^{2+} in the regulation of parathyroid gland function during CRF. In addition, it is interesting to note that the horse (another mammal with high basal Ca^{2+} that develops hypercalcemia during CRF) behaves similarly (31).

In conclusion, a difference in the PTH response to hypocalcemia has been found in rabbits after exposure to either acute or chronic hypercalcemia. After acute hypercalcemia, an attenuated PTH response to hypocalcemia, similar to that previously described in dogs, has been identified. Chronic hypercalcemia, however, did not influence the PTH response to hypocalcemia. These results suggest that rabbit parathyroid gland function is unchanged after chronic hypercalcemia.

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References

- 1 Brent GA, Leboff MS, Seely EW, Conlin PR & Brown EM. Relationship between the concentration and rate of change of calcium and serum intact parathyroid hormone levels in normal humans. *Journal of Clinical Endocrinology and Metabolism* 1988 **67** 944–950.
- 2 Mayer GP & Hurst JG. Sigmoidal relationship between parathyroid hormone secretion rate and plasma calcium concentration in calves. *Endocrinology* 1978 **102** 1036–1042.
- 3 Sánchez J, Aguilera-Tejero E, Estepa JC, Almadén Y, Rodríguez M & Felsenfeld AJ. A reduced PTH response to hypocalcemia after a short period of hypercalcemia: a study in dogs. *Kidney International* 1996 **50** (Suppl 57) S18–S22.
- 4 Habener JF, Kemper B & Potts JR. Calcium-dependent intracellular degradation of parathyroid hormone: a possible mechanism for the regulation of hormone stores. *Endocrinology* 1975 **97** 431–441.
- 5 Chu LLH, MacGregor RR, Anast CS, Hamilton JW & Cohn DV. Studies on the biosynthesis of rat parathyroid hormone and parathyroid hormone: adaptation of the parathyroid gland to dietary restriction of calcium. *Endocrinology* 1973 **93** 915–924.
- 6 Brossard J-H, Whittom S, LePage R & D'Amour P. Carboxyl-terminal fragments of parathyroid hormone are not secreted

- preferentially in primary hyperparathyroidism as they are in other hypercalcemic conditions. *Journal of Clinical Endocrinology and Metabolism* 1993 **77** 413–419.
- 7 Russell J, Lettieri D & Sherwood LM. Direct regulation by calcium of cytoplasmic messenger ribonucleic acid coding for pre-parathyroid hormone in isolated bovine parathyroid cells. *Journal of Clinical Investigation* 1983 **72** 1851–1855.
 - 8 Yamamoto M, Igarashi T, Muramatsu M, Fukagawa M, Motokura T & Ogata E. Hypocalcemia increases and hypercalcemia decreases the steady-state level of parathyroid hormone messenger RNA in the rat. *Journal of Clinical Investigation* 1989 **83** 1053–1056.
 - 9 Butters RR, Chattopadhyay N, Nielsen P, Smith CP, Mithal A, Kifor O *et al.* Cloning and characterization of a calcium-sensing receptor from the hypercalcemic New Zealand White rabbit reveals unaltered responsiveness to extracellular calcium. *Journal of Bone and Mineral Research* 1997 **12** 568–579.
 - 10 Warren HB, Lausen NCC, Segre GV, El-Hajj G & Brown EM. Regulation of calciotropic hormones in vivo in the New Zealand White rabbit. *Endocrinology* 1989 **125** 2683–2690.
 - 11 Eddy AE, Falk RJ, Sibley RK & Hostetter TH. Subtotal nephrectomy in the rabbit: A model of chronic hypercalcemia, nephrolithiasis, and obstructive nephropathy. *Journal of Laboratory and Clinical Medicine* 1986 **107** 508–516.
 - 12 Tennant B, Lowe JE & Tasker JB. Hypercalcemia and hypophosphatemia in ponies following bilateral nephrectomy. *Proceedings of the Society for Experimental Biology and Medicine* 1981 **167** 365–368.
 - 13 Tvedegaard E. Renal function and 1 α -hydroxyvitamin D₃ in rabbits with normal renal function and renal insufficiency. *Scandinavian Journal of Urology and Nephrology* 1983 **17** 373–376.
 - 14 Yu L, Pragay DA, Chang D & Wicher K. Biochemical parameters of normal rabbit serum. *Clinical Biochemistry* 1979 **12** 83–87.
 - 15 Torrance AG & Nachreiner R. Human-parathormone assay for use in dogs: validation, sample handling studies and parathyroid function testing. *American Journal of Veterinary Research* 1989 **50** 1123–1127.
 - 16 Jara A, Bover J, Lavigne J & Felsenfeld A. Comparison of two parathyroid hormone assays for the rat: the new immunoradiometric and the older competitive binding assay. *Journal of Bone and Mineral Research* 1994 **10** 1629–1633.
 - 17 Estepa JC, Aguilera-Tejero E, Mayer-Valor R, Almadén Y, Felsenfeld AJ & Rodríguez M. Measurement of parathyroid hormone in horses. *Equine Veterinary Journal* 1998 **30** 476–481.
 - 18 Cloutier M, Gascon-Barre M & D'Amour P. Chronic adaptation of dog parathyroid function to a low-calcium–high-sodium–vitamin D-deficient diet. *Journal of Bone and Mineral Research* 1992 **7** 1021–1028.
 - 19 Cloutier M, Brossard JH, Gascon-Barre M & D'Amour P. Lack of involution of hyperplastic parathyroid glands in dogs: adaptation via a decrease in the calcium stimulation set point and a change in secretion profile. *Journal of Bone and Mineral Research* 1994 **9** 621–629.
 - 20 Mayer GP, Keaton JA, Hurst JG & Habener JE. Effects of plasma calcium concentration on the relative proportion of hormone and carboxyl fragments in parathyroid venous blood. *Endocrinology* 1979 **104** 1778–1784.
 - 21 Ritchie CK, Cohn DV, Maercklein PB & Fitzpatrick LA. Individual parathyroid cells exhibit cyclic secretion of parathyroid hormone and chromogranin-A (as measured by a novel sequential hemolytic plaque assay). *Endocrinology* 1992 **31** 2638–2642.
 - 22 Fasciottio BH, Gorr SU, DeFranco DJ, Levine M & Cohn DV. Pancreastatin, a presumed product of chromogranin A (secretory protein-1) processing inhibits secretion from porcine parathyroid cells in culture. *Endocrinology* 1989 **125** 1617–1622.
 - 23 Drees BM & Hamilton JW. Pancreastatin and bovine parathyroid cell secretion. *Bone and Mineral* 1992 **17** 335–346.
 - 24 Fujii Y, Moreira JE, Orlando C, Maggi M, Aurbach GD, Brandi ML *et al.* Endothelin as autocrine factor in the regulation of parathyroid cells. *PNAS* 1991 **88** 4235–4239.
 - 25 Bourdeau A, Sourberbielle JC, Bonnet P, Herviaux P, Sachs CH & Lieberherr M. Phospholipase A₂ action and arachidonic acid metabolism in calcium-mediated parathyroid hormone secretion. *Endocrinology* 1992 **130** 1339–1344.
 - 26 Sun F, Ritchie C, Maercklein P & Fitzpatrick LA. Heterogeneous response to calcium by individual parathyroid cells. *Journal of Clinical Investigation* 1993 **91** 595–601.
 - 27 Conlin PR, Fajtova VT, Mortensen RM, Leboff MS & Brown EM. Hysteresis in the relationship between serum ionized calcium and intact parathyroid hormone during recovery from induced hyper- and hypocalcemia in normal humans. *Journal of Clinical Endocrinology and Metabolism* 1989 **69** 593–599.
 - 28 Silver J, Russell J, Lettieri D & Sherwood LM. Regulation by vitamin D metabolites of messenger ribonucleic acid for preparathyroid hormone in isolated bovine parathyroid cells. *PNAS* 1985 **82** 4270–4273.
 - 29 Russell J, Lettieri D & Sherwood LM. Suppression by 1,25(OH)₂D₃ of the pre-parathyroid hormone gene. *Endocrinology* 1986 **119** 2864–2866.
 - 30 Naveh-Manly T & Silver J. Regulation of parathyroid hormone gene expression by hypocalcemia, hypercalcemia, and vitamin D in the rat. *Journal of Clinical Investigation* 1990 **86** 1313–1319.
 - 31 Aguilera-Tejero E, Estepa JC, López I, Bas S & Rodríguez M. Polycystic kidneys as a cause of chronic renal failure and secondary hypoparathyroidism in a horse. *Equine Veterinary Journal* 2000 **32** 167–169.

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