Both duration and degree of hypercalcemia influence the reduced parathyroid hormone response to hypocalcemia after hypercalcemia

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Abstract

The stimulation of parathyroid hormone (PTH) secretion by hypocalcemia is reduced when hypocalcemia is preceded by hypercalcemia. The present study investigates whether the duration and degree of hypercalcemia influence the reduced PTH response to hypocalcemia after hypercalcemia. In addition, the implication of the arachidonic acid (AA) signaling pathway in this effect is evaluated. The PTH response to hypocalcemia has been studied in a control group and in four groups of rabbits subjected to hypercalcemia for different periods of time (between 30 and 120 min) and at two levels of hypercalcemia (1.9 and 2.1 mM). AA levels have been measured in parathyroid glands from rabbits subjected to hyper- and hypocalcemia. When compared with controls, rabbits that had been hypercalcemic (2.1 mM) for 2 h showed a markedly attenuated PTH response to hypocalcemia (50% of normal PTHmax), rabbits that had been in

hypercalcemia (2.1 mM) for 75 min had an intermediate PTH response to hypocalcemia (70% of normal PTHmax) and rabbits that had been subjected to either 30 min hypercalcemia of 2.1 mM or 120 min hypercalcemia of 1.9 mM had a normal PTH response to hypocalcemia. AA levels increased in hypercalcemia and decreased in hypocalcemia; however, no differences were observed at either calcium level in short-time (30 min) versus long-time (120 min) hypercalcemia. In conclusion, the attenuated PTH response to hypocalcemia after hypercalcemia is dependent on both the period of time that the parathyroid glands have been exposed to hypercalcemia and the degree of hypercalcemia. In addition, this reduced PTH response does not seem to be related to changes in the AA signaling pathway.

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Introduction

A reduced parathyroid hormone (PTH) response to hypocalcemia after a short period of hypercalcemia has previously been reported in cows (Blum *et al.* 1981), dogs (Sanchez *et al.* 1996) and rabbits (Bas *et al.* 2002). Moreover, the PTH–calcium curve after hypercalcemia has been studied in detail in dogs and rabbits. In both studies the animals were subjected to a hypercalcemic clamp (blood Ca^{2+} was increased by 0.4 mM) for 2 h and then their parathyroid glands were stimulated by hypocalcemia. When compared with animals in which hypocalcemia was initiated from basal Ca^{2+} levels, the PTH response to hypocalcemia was less for the same concentration of Ca^{2+} when the reduction in Ca^{2+} was initiated from hypercalcemia compared with that from a normal plasma calcium concentration (Sanchez *et al.* 1996, Bas *et al.* 2002).

Since both prolonged (2 months) and very short (10 min) periods of hypercalcemia have been reported not

to modify the PTH response to hypocalcemia (Blum *et al.* 1981, Bas *et al.* 2002), a time-frame should exist for the attenuation of the PTH response after hypercalcemia to take place. In addition, we hypothesize that the inhibitory effect of hypercalcemia on a subsequent hypocalcemic stimulus could also be related to the degree to which calcium is elevated.

The blunted PTH response to hypocalcemia after hypercalcemia may indicate that PTH secretion is disturbed but it could also be interpreted as a physiological mechanism – the parathyroid cells may have some kind of ‘memory’ which modulates their response in the face of previous events. The latter hypothesis is supported by other characteristics of the PTH– Ca^{2+} relationship (e.g. hysteresis). The phenomenon of hysteresis is that, for the same Ca^{2+} level, PTH values are higher when inducing hypocalcemia than when recovering from hypocalcemia. Similarly, PTH concentrations are lower when inducing hypercalcemia than when recovering from hypercalcemia (Conlin *et al.* 1989, Grant *et al.* 1990). It has been

suggested that the hysteresis of the PTH–Ca²⁺ curve is a defence mechanism intended to foresee the future evolution of Ca²⁺ levels (Aguilera-Tejero *et al.* 1996).

The mechanisms by which hypercalcemia attenuates the PTH response to hypocalcemia are unknown. Hypercalcemia has been reported to modify PTH metabolism (Chu *et al.* 1973, Habener *et al.* 1975), to influence PTH gene transcription and to decrease PTH mRNA levels (Russel *et al.* 1983, Yamamoto *et al.* 1989). Hypercalcemia is also known to modify the secretory profile of PTH and its fragments – secretion of carboxyterminal PTH is less suppressed than secretion of intact PTH when extracellular Ca²⁺ is elevated (Mayer *et al.* 1979, Cloutier *et al.* 1992, 1994).

Since the reduced response to hypocalcemia is evident when the parathyroid glands have been exposed to hypercalcemia for only 2 h (Sanchez *et al.* 1996, Bas *et al.* 2002), it is likely that post-translational mechanisms are involved. We hypothesize that changes in intracellular sensing mechanisms triggered by the action of Ca²⁺ on the calcium receptor (CaR) could explain this phenomenon. We and others have shown that the phospholipase A₂–arachidonic acid (PLA₂–AA) pathway is a key signaling system which mediates the inhibition of PTH secretion in response to increases in extracellular Ca²⁺ (Bourdeau *et al.* 1992, Almaden *et al.* 2002). Therefore, AA is a likely candidate to be involved in the attenuation of the PTH response after hypercalcemia.

The objectives of the work reported here were (1) to determine the time of exposure to hypercalcemia required to observe a reduced PTH response to hypocalcemia; (2) to investigate the degree of hypercalcemia (i.e. the elevation in extracellular Ca²⁺) necessary to elicit a reduced PTH response to hypocalcemia; and (3) to study the influence that an intracellular signaling pathway (AA) may have on the reduced PTH response to hypocalcemia after hypercalcemia.

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 housed individually, had free access to water, and a commercial diet containing 1.2% Ca and 0.6% P was available *ad libitum*. The rabbits were randomly assigned to any of the following groups. Group I (*n*=10): these animals were used as a control to obtain the PTH–Ca²⁺ curve in normocalcemic rabbits (plasma Ca²⁺=1.7 mM). Group II (*n*=10): in this group plasma Ca²⁺ was progressively increased from its baseline value of 1.7 mM to 2.1 mM during a period of 30 min. Immediately after reaching 2.1 mM, calcium was progressively decreased to a level below 1 mM during a 60-min period. Group III (*n*=9): in these rabbits, plasma Ca²⁺ was

increased from its baseline value (1.7 mM) to 2.1 mM during the first 30 min and then was maintained elevated for an additional period of 45 min. Subsequently hypocalcemia was induced as in group II. Group IV (*n*=9): hypercalcemia was induced as in groups II and III but the hypercalcemic clamp was extended to a period of 90 min before the hypocalcemic stimulation. Group V (*n*=7): these rabbits were also subjected to a 90-min hypercalcemic clamp but plasma Ca²⁺ was elevated only 0.2 mM. Thus, during the first 30 min plasma Ca²⁺ was raised from 1.7 mM to 1.9 mM, and then was clamped for an additional 90 min at 1.9 mM. Subsequently, hypocalcemia was induced as in groups II–IV.

Five additional experimental groups were studied to determine AA production by parathyroid tissue. Group VI (*n*=5): in these rabbits plasma Ca²⁺ was elevated as in group II. At the end of 30 min hypercalcemia, the parathyroid glands were removed. Group VII (*n*=5): these rabbits were subjected to hypercalcemia for 30 min, followed by 60 min hypocalcemia (as in group II). At the end of the hypocalcemic stimulation, parathyroidectomy was performed. Group VIII (*n*=6): in these rabbits plasma Ca²⁺ was elevated following the same protocol as in group IV and at the end of the 90-min hypercalcemic clamp the parathyroid glands were removed. Group IX (*n*=5): rabbits followed the same protocol as group IV and parathyroidectomy was performed at the end of the 60-min hypocalcemic stimulus. Group X (*n*=7): these rabbits served as a control group for AA content in parathyroid tissue; the parathyroid glands were obtained without modifying plasma Ca²⁺.

PTH–Ca²⁺ curves

PTH–Ca²⁺ curves were obtained by i.v. infusion of disodium EDTA. In all experimental groups, rabbits were anesthetized using a single dose of ketamine (40 mg/kg) and midazolam (1 mg/kg) administered before starting the experiments. Rabbits in groups IV, V, VIII and IX received an additional dose of ketamine (20 mg/kg) and midazolam (0.5 mg/kg) at 100 min to prolong anesthesia until the end of the experiments. The marginal auricular vein and the central auricular artery were cannulated with 24 G catheters. The venous port was used for CaCl₂ and EDTA infusion and the arterial side for blood sampling. The protocols for induction of hyper- and hypocalcemia are set out below.

Hypercalcemia In groups II, III, IV, VI, VII, VIII and IX, hypercalcemia was achieved by i.v. infusion of CaCl₂ at a mean rate of 1.5 mEq/kg/h during 30 min. When a calcium clamp was performed, hypercalcemia was maintained by infusing CaCl₂ at a rate of 1.2 mEq/kg/h during 45 min (group III) or 90 min (groups IV, VIII and IX). In group V, hypercalcemia was induced during 30 min and

maintained during 90 min by infusing 0.75 mEq/kg/h of CaCl_2 .

Hypocalcemia In group I, hypocalcemia was induced by an EDTA infusion which was initiated at a rate of 50 mg/kg/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/h at the end of the experiment (35 min). In groups II, III, IV, VI, VII, VIII and IX, hypocalcemia was induced, first by reducing the dose of CaCl_2 during 15 min and then by infusing EDTA at an initial rate of 25 mg/kg/h which was progressively increased to 240 mg/kg/h at the end of the experiment (60 min). In group V, hypocalcemia was achieved, first by reducing the dose of CaCl_2 over a 10-min period and then by EDTA infusion starting at 25 mg/kg/h and finishing at 180 mg/kg/h.

To avoid interference associated with volume loading, the rate of administration of fluids was adjusted to be identical in all groups during induction of hyper- and hypocalcemia. During the hypercalcemic clamp volume infusion rates were also very similar ranging from 0.06 ml/kg/min (group V) to 0.07 ml/kg/min (group III). Moreover, the sodium load was identical in all experimental groups.

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) after which plasma was frozen at -70°C . PTH was measured on plasma samples within 3 months of collection using an immunoradiometric assay (Allegro Intact PTH, Nichols, San Juan Capistrano, CA, USA) which has previously been validated for quantitation of rabbit PTH (Warren *et al.* 1989). Plasma phosphate and magnesium concentrations were measured using routine spectrophotometric techniques (Sigma, St Louis, MO, USA).

During the experiments, blood samples were obtained at predetermined time intervals. Since Ca^{2+} values were not identical in all rabbits at the time of sampling, the following procedure was used to obtain the PTH- Ca^{2+} curve at standardized Ca^{2+} levels (Aguilera-Tejero *et al.* 1996, Bas *et al.* 2002). First, individual PTH- Ca^{2+} curves were constructed by adjusting the PTH and Ca^{2+} values of every rabbit to a sigmoidal equation. The PTH values used for the hypercalcemic part of the curve were those recorded during the hypocalcemic stimulation (when calcium was decreased from 2.1 or 1.9 mM to 1.7 mM). The PTH concentrations at standardized Ca^{2+} levels (from $\text{Ca}^{2+}=2.1$ mM to $\text{Ca}^{2+}=1$ 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.

AA measurement

AA was measured in parathyroid tissue. Rabbits from groups VI–X were anesthetized as described above. In groups VI–IX hyper- or hypocalcemia was induced using the above mentioned protocols. The external parathyroid glands were surgically exposed and were then carefully removed without thyroid tissue. The time required to complete parathyroidectomy was less than 5 min.

Glands were dissected free of fat and connective tissue and were placed in individual wells submerged in ice. Parathyroid tissue was homogenized with a glass homogenizer in a solution containing 50 mM Tris-HCl (pH 7.5 at 25°C), 0.2 mM EDTA, and 0.5 mM dithiothreitol. AA was quantified by gas chromatography (model 5890-A; Hewlett Packard, Avondale, PA, USA) as described elsewhere (Cardenas *et al.* 1994). The coefficient of variation for AA quantification was 2.5%. The protein content of the tissue samples was determined using the Bradford method (Bradford 1976).

Statistics

For the intra- or intergroup comparison of three or more samples, repeated analysis of variance (ANOVA) was used. If the ANOVA showed statistical differences, a *post hoc* test, the Scheffe test, was used to determine differences. A *P* value <0.05 was considered significant. Results are expressed as the mean $\pm$ S.E.

Results

Ca^{2+} and PTH values

The time course for plasma Ca^{2+} and PTH in groups I, II, III, IV and V is presented in Fig. 1. No significant differences in baseline Ca^{2+} were detected between groups. In groups II–V Ca^{2+} was elevated as scheduled. Thus Ca^{2+} values before induction of hypocalcemia were 2.12 ± 0.03 mM (group II), 2.13 ± 0.06 mM (group III), 2.08 ± 0.02 mM (group IV) and 1.88 ± 0.02 mM (group V). All these Ca^{2+} levels were significantly higher than baseline. In rabbits from group V the Ca^{2+} concentration before induction of hypocalcemia (1.88 ± 0.02 mM) was lower ($P<0.05$) than in groups II–IV. During hypocalcemia there was a parallel decrease in Ca^{2+} in all groups, reaching final values that ranged between 0.96 and 1.09 mM (Fig. 1A).

No significant differences were found in baseline PTH in groups I–V. Hypercalcemia resulted in a decrease in PTH concentration and the values measured before starting hypocalcemia were: 5.7 ± 0.6 pg/ml (group II), 2.9 ± 1.1 pg/ml (group III), 4.8 ± 0.9 pg/ml (group IV) and 10.6 ± 2.3 pg/ml (group V). All these values were significantly lower than baseline and the PTH value in group V was higher than in groups II–IV ($P<0.05$).

Figure 1 The time course of (A) Ca^{2+} and (B) PTH in groups I to V. Values are means $\pm$ S.E. (when the standard error bar is not present, it was too small to draw). Group I, normocalcemic rabbits ($\square$); group II, rabbits subjected to 30 min hypercalcemia, 2.1 mM ($\bullet$); group III, rabbits subjected to a 45-min hypercalcemic clamp, 2.1 mM ($\circ$); group IV, rabbits subjected to a 90-min hypercalcemic clamp, 2.1 mM ($\triangle$); group V, rabbits subjected to a 90-min hypercalcemic clamp, 1.9 mM ($\blacktriangle$).

During hypocalcemia PTH concentration rose in group I to a maximum (PTHmax) of 99.7 ± 4.2 pg/ml. PTHmax in groups II (103.3 ± 7.6 pg/ml) and V (108.3 ± 9.2 pg/ml) was not different from group I. Rabbits from group III had a PTHmax (68.7 ± 8.1 pg/ml) which was lower ($P < 0.05$) than groups I, II and V. Finally the PTHmax in

group IV was significantly lower (36.4 ± 7.6 pg/ml) than in all the other groups (Fig. 1B).

A summary of the changes in Ca^{2+} and PTH in the experimental groups I, II, III, IV and V is shown in Table 1.

Phosphate and magnesium concentrations are presented in Table 2. Mean baseline P in all groups ranged from 1.23 to 1.28 mM and did not change in any group throughout the experiments. Mean baseline Mg, which ranged from 0.97 to 0.99 mM, did not change during any of the protocols for induction of hypercalcemia. EDTA infusion resulted in a progressive decrease in Mg that was parallel to the decline in Ca^{2+} . Changes in Mg during EDTA administration were similar in all experimental groups.

PTH- Ca^{2+} curves

The PTH- Ca^{2+} curves in groups I–V are shown in Fig. 2. In group I, at baseline Ca^{2+} of 1.7 mM, the PTH concentration was 40.1 ± 5.3 pg/ml. Hypocalcemia caused an increase in PTH concentration up to a maximum of 103.1 ± 3.2 pg/ml which was achieved with a Ca^{2+} concentration of 1.25 mM. PTH values remained stabilized at Ca^{2+} levels between 1.25 and 1 mM. In groups II, III and IV in which the hypocalcemic stimulus was initiated from hypercalcemia, the minimal PTH concentration was recorded at a Ca^{2+} concentration of 2.1 mM. At this Ca^{2+} level, PTH values were below 5 pg/ml and no significant differences were found between groups. In the hypocalcemic part of the curve, no significant differences between groups I and II were found at any Ca^{2+} concentration. In group III, PTH values were significantly lower than in group I at all Ca^{2+} values below 1.6 mM. PTH values in group IV were significantly lower than in groups I and II at all Ca^{2+} values below 1.65 mM. In addition, PTH values at Ca^{2+} levels below 1.65 mM were lower in group IV than in group III ($P < 0.05$). The PTH- Ca^{2+} curve in group V shows that PTH values during hypercalcemia (between Ca^{2+} 1.7 and 1.85 mM) were similar to groups II and III. At any Ca^{2+} concentration between 1.7 and 1 mM, PTH levels were almost identical in groups I and V.

Table 1 Summary of Ca^{2+} and PTH values in the experimental groups I to V

Group	Basal Ca^{2+} (mM)	Ca^{2+} (mM) at the end of hypercalcemia	Ca^{2+} (mM) at the end of hypocalcemia	Basal PTH (pg/ml)	PTH (pg/ml) at the end of hypercalcemia	PTH (pg/ml) at the end of hypocalcemia
I	1.69 ± 0.02^a	—	1.09 ± 0.07^a	40.10 ± 5.33^a	—	99.70 ± 4.25^a
II	1.68 ± 0.01^a	2.12 ± 0.03^a	1.09 ± 0.05^a	36.79 ± 4.47^a	5.72 ± 0.676^a	103.33 ± 7.57^a
III	1.70 ± 0.02^a	2.13 ± 0.06^a	1.09 ± 0.05^a	31.68 ± 5.85^a	2.87 ± 1.07^a	68.69 ± 8.05^b
IV	1.70 ± 0.02^a	2.08 ± 0.02^a	0.96 ± 0.03^a	25.22 ± 4.81^a	4.84 ± 0.88^a	36.44 ± 7.65^c
V	1.69 ± 0.03^a	1.88 ± 0.02^b	1.01 ± 0.05^a	36.58 ± 5.73^a	10.63 ± 2.30^b	108.28 ± 9.20^a

Within each column, superscripts with different letters indicate significant differences between groups ($P < 0.05$).

Table 2 Summary of phosphate (P) and magnesium (Mg) values in the experimental groups I to V

Group	Basal P (mM)	P (mM) at the end of hypercalcemia	P (mM) at the end of hypocalcemia	Basal Mg (mM)	Mg (mM) at the end of hypercalcemia	Mg (mM) at the end of hypocalcemia
I	1.23 ± 0.09	—	1.31 ± 0.09	0.99 ± 0.05	—	0.41 ± 0.04 ^a
II	1.24 ± 0.12	1.29 ± 0.11	1.30 ± 0.16	0.98 ± 0.04	0.99 ± 0.04	0.43 ± 0.05 ^a
III	1.27 ± 0.09	1.23 ± 0.12	1.30 ± 0.11	0.98 ± 0.05	0.99 ± 0.05	0.47 ± 0.05 ^a
IV	1.28 ± 0.05	1.28 ± 0.05	1.26 ± 0.07	0.97 ± 0.06	1.00 ± 0.08	0.47 ± 0.06 ^a
V	1.28 ± 0.08	1.29 ± 0.05	1.26 ± 0.07	0.98 ± 0.07	0.97 ± 0.04	0.40 ± 0.05 ^a

No significant differences between groups were found at any sampling time. ^a $P < 0.05$ when compared with baseline.

As described above, the PTH values used to construct the PTH- Ca^{2+} curves in groups II–V were those obtained during hypocalcemic stimulation from hypercalcemia. However, in the hypercalcemic part of the PTH- Ca^{2+} curve two sets of PTH values were recorded for each Ca^{2+} concentration – PTH during induction of hypercalcemia from normocalcemia (first 30 min of experiment) and PTH during induction of hypocalcemia from hypercalcemia (last 60 min of experiment). These two sets of PTH values are shown in Fig. 3. In rabbits from groups II and V, PTH values during recovery from hypercalcemia were higher than PTH values during induction of hypercalcemia ($P < 0.05$ at $\text{Ca}^{2+} = 1.8$ mM), i.e. hysteresis of the PTH- Ca^{2+} curve was observed (Fig. 3A and B). However, in rabbits from groups III and IV no difference was found between PTH values during induction of and recovery from hypercalcemia (Fig. 3C and D).

AA measurements

AA levels in the parathyroid glands of rabbits in groups VI–X are presented in Fig. 4. AA production by

parathyroid tissue was significantly higher ($P = 0.03$) during hypercalcemia (group VI = $9.2 \pm 2.3 \times 10^{-2}$ $\mu\text{g}/\mu\text{g}$ protein and group VIII = $7.5 \pm 1.7 \times 10^{-2}$ $\mu\text{g}/\mu\text{g}$ protein) than during hypocalcemia (group VII = $3.9 \pm 0.9 \times 10^{-2}$ $\mu\text{g}/\mu\text{g}$ protein, group IX = $2.4 \pm 0.5 \times 10^{-2}$ $\mu\text{g}/\mu\text{g}$ protein). No significant differences were detected between groups VI and VIII or between groups VII and IX. Normocalcemic rabbits had intermediate AA levels (group X = $6.6 \pm 1.4 \times 10^{-2}$ $\mu\text{g}/\mu\text{g}$ protein) in their parathyroid glands (Fig. 4A).

As illustrated in Fig. 4B, in the rabbits in which AA was measured, plasma PTH concentration changed as described above for groups II and IV. Thus, at elevated Ca^{2+} (2.1 mM) PTH values were very low and similar in groups VI (5.7 ± 0.7 pg/ml) and VIII (4.7 ± 0.7 pg/ml). Decreasing Ca^{2+} concentration resulted in an increase in PTH levels. At low Ca^{2+} (1 mM), there was a significant difference in PTH values between groups VII (103.3 ± 7.6 pg/ml) and IX (38.3 ± 5.6 pg/ml).

Discussion

The objective of the present study was to investigate the circumstances that influence the reduced PTH response to hypocalcemia after the parathyroid glands have been subjected to hypercalcemia. Our results show that the attenuated PTH response to hypocalcemia is dependent on both the time the parathyroid glands have been exposed to hypercalcemia and the degree of hypercalcemia. In addition, this reduced PTH response does not seem to be related to changes in the AA signaling pathway.

The PTH- Ca^{2+} curve obtained in normal rabbits is similar to that which has previously been reported elsewhere (Warren *et al.* 1989; Bas *et al.* 2002). Likewise, the 90-min hypercalcemic clamp resulted in an attenuated PTH response to hypocalcemia, comparable to that which has previously been described in dogs and rabbits (50% decrease in PTHmax) (Sanchez *et al.* 1996, Bas *et al.* 2002). The 45-min hypercalcemic clamp resulted in a PTH response that was intermediate between those of rabbits in which hypocalcemia was initiated from normal Ca^{2+} and rabbits that had been subjected to a 90-min

Figure 2 The PTH- Ca^{2+} curve in groups I–V. Values are means $\pm$ S.E. (when the standard error bar is not present, it was too small to draw). Where the arrows start indicates that PTH values are significantly lower than the PTH concentration registered in group I for the same Ca^{2+} level. Group I ($\square$); group II ($\bullet$); group III ($\circ$); group IV ($\triangle$); group V ($\blacktriangle$).

Figure 3 The PTH- Ca^{2+} curve in groups II (A), V (B), III (C) and IV (D) during induction of (solid line) and recovery from (dashed line) hypercalcemia. * $P < 0.05$ significant differences in PTH concentration, for the same Ca^{2+} value, during induction of and recovery from hypercalcemia.

hypercalcemic clamp. The PTHmax of these rabbits was approximately 70% of the PTHmax of normal rabbits. Induction of hypercalcemia in the 30 min prior to the hypocalcemic stimulus did not modify the PTH response to hypocalcemia.

These results indicate that an attenuated PTH response to hypocalcemia requires more than 30 min of hypercalcemia, and the longer the time in hypercalcemia the more attenuated the PTH response to hypocalcemia. However, this process must show an inflexion at some time point, since it has also been demonstrated that rabbits become adapted to chronic (2 months) hypercalcemia and that the PTH- Ca^{2+} curve in chronic hypercalcemic rabbits is almost identical to the PTH- Ca^{2+} curve in normal rabbits (Bas *et al.* 2002).

The period of time in hypercalcemia is not the only factor involved in the attenuation of the subsequent response to hypocalcemia, since in parathyroid glands exposed for 90 min to Ca^{2+} levels of 1.9 mM (only 0.2 mM increase over basal Ca^{2+}) there was no reduced

PTH response to hypocalcemia. Thus, Ca^{2+} needs to be increased by >0.2 mM to observe a decrease in the PTH response to hypocalcemia.

During EDTA infusion there was a progressive decrease in Mg that was parallel to the decline in Ca^{2+} . Although hypomagnesemia is known to stimulate PTH secretion, the decrease in Mg at a time when the parathyroid cells are subjected to a much more potent stimulus (hypocalcemia) does not influence the PTH response (López 2002). In addition, changes in Mg were similar in all groups and therefore cannot explain the differences found in the PTH response.

It is known that for the same Ca^{2+} level PTH values are higher when recovering from hypercalcemia than when inducing hypercalcemia – hysteresis of the PTH- Ca^{2+} curve (Conlin *et al.* 1989, Grant *et al.* 1990). In their studies Conlin *et al.* and Grant *et al.* increased the plasma Ca^{2+} by 0.15 mM during 90 min in healthy humans and recorded higher PTH values in the subsequent hypocalcemia than those measured during induction of

Figure 4 (A) Arachidonic acid (AA) content (in $\mu\text{g}/\mu\text{g}$ protein) in parathyroid tissue from rabbits at three levels of extracellular Ca^{2+} . (B) Plasma PTH concentration (in pg/ml) in rabbits at three levels of extracellular Ca^{2+} . * $P < 0.05$, significant differences between rabbits subjected to short-time hypercalcemia and to a hypercalcemic clamp. Solid bars indicate rabbits subjected to hypercalcemia for 30 min (groups VI and VII) and open bars indicate rabbits subjected to a 90-min hypercalcemic clamp (groups VIII and IX). Gray bars indicate rabbits in which plasma Ca^{2+} was not modified (group X).

hypercalcemia. The same phenomenon was observed in our study in group V, where Ca^{2+} was elevated by only 0.2 mM for 120 min, and, to a lesser extent, in group II, where Ca^{2+} was elevated by 0.4 mM for only 30 min. However, in groups III and IV, where Ca^{2+} was increased by 0.4 mM during 75 and 120 min respectively, no hysteresis was recorded. Thus, hysteresis of the PTH- Ca^{2+} curve seems to be dependent on both the level to which Ca^{2+} is elevated and the time elapsed in hypercalcemia.

It is also interesting to point out that the PTH response to hypocalcemia in the hypercalcemic range of the PTH- Ca^{2+} curve seems to be related to the final PTH response to hypocalcemia – hysteresis was only found in the two groups (II and V) that reached the same PTHmax as the control

group. However, this situation is somewhat different in chronic hypercalcemia. Rabbits subjected to hypercalcemia for 2 months have been shown to have the same PTHmax in response to hypocalcemic stimulation as normal rabbits; however, no hysteresis was found in the hypercalcemic part of their PTH- Ca^{2+} curve (Bas *et al.* 2002).

The reasons for the reduced PTH response to hypocalcemia after a relatively short period of hypercalcemia are not clear. Hypercalcemia has been shown to inhibit PTH biosynthesis (Russell *et al.* 1983, Yamamoto *et al.* 1989). However, the rapid regulation described in this paper is probably best explained by changes in the amount of PTH available for secretion, which is controlled by a Ca^{2+} -dependent degradation process. High levels of extracellular Ca^{2+} have been shown to promote intracellular PTH degradation both *in vivo* (Chu *et al.* 1973) and *in vitro* (Habener *et al.* 1975); thus an increase in intracellular PTH metabolism could be a major factor in the reduced PTH response to hypocalcemia found in groups III and IV. 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 carboxyterminal PTH is less suppressed (Mayer *et al.* 1979, Cloutier *et al.* 1992, 1994). In addition to the secretion of intact PTH and its fragments, several secretory products of the parathyroid glands such as chromogranin A and its metabolites (Fasciottio *et al.* 1989, Drees & Hamilton 1992, Ritchie *et al.* 1992) and endothelin 1 (Fujii *et al.* 1991) could play a role in the inhibition of PTH secretion after acute hypercalcemia.

Any kind of post-transcriptional regulation that would serve to explain the inhibitory effect of hypercalcemia on a subsequent hypocalcemic stimulus should be related to changes in intracellular signaling mechanisms triggered by the action of Ca^{2+} on the CaR. In this context, AA participates in a major signaling pathway within the parathyroid glands (Bourdeau *et al.* 1992, Almaden *et al.* 2000, 2002). Our results show predictable changes in AA concentration in the parathyroid glands with hypo- and hypercalcemia, AA levels being significantly increased during hypercalcemia when compared with hypocalcemia. However, the disparate PTH response to hypocalcemia after short-time (30 min) and long-time (120 min) hypercalcemia cannot be explained by changes in AA content in the parathyroid glands. Thus, it appears that changes in signal transduction via the PLA_2 -AA pathway are not the reason for the uneven PTH response to hypocalcemia after different periods of time in hypercalcemia.

In conclusion, the attenuated PTH response to hypocalcemia after hypercalcemia is dependent on both the time the parathyroid glands have been exposed to hypercalcemia and the degree of hypercalcemia. In addition, this reduced PTH response does not seem to be related to changes in AA concentration.

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