INVITED COMMENTARY

Effects of acute and chronic hypercalcemia on parathyroid function and circulating parathyroid hormone molecular forms

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Bas *et al.* (1), in this issue, have demonstrated that acute hypercalcemic clamp over 2 h in rabbits reduces the subsequent intact (I) parathyroid hormone (PTH) response to hypocalcemia by 50%, while chronic hypercalcemia, secondary to experimental renal failure in the same animal model, fails to reduce the I-PTH response to hypocalcemia. These results raise questions about the acute and chronic influence of calcium concentration on parathyroid function and on circulating PTH molecular forms. To understand and appropriately discuss these differences, it is necessary to review various aspects of PTH physiology.

Circulating PTH is immunoheterogenous. Under normocalcemic conditions, it is composed of 20% PTH(1–84), the biologically active form of the hormone on the PTH/PTHrP receptor, and of 80% carboxyl-terminal (C) fragments, considered until recently to be biologically inactive (2, 3). The main circulating C-PTH fragments observed during the peripheral metabolism of ¹²⁵I-bovine (b) PTH(1–84) in rats start at positions 34, 37 and 39 (4) of the bPTH structure. Porcine (p) parathyroid cells have been demonstrated to secrete pPTH fragments starting at positions 34 and 37 (5). Studies in humans have also outlined the existence of smaller C-PTH fragments (6) and, more recently, of larger C-PTH fragments with a partially preserved aminoterminal structure (7), called non-(1–84)PTH. These non-(1–84)PTH molecular form or forms have been described in humans during the analysis of normal and abnormal serum HPLC profiles using Nichol's I-PTH assay (7), the same assay as that employed by Bas *et al.* (1) in their study. Most I-PTH assays have since been demonstrated to also react with non-(1–84)PTH (8). Non-(1–84)PTH accounts for 10% of C-PTH fragments and for 20% of I-PTH immunoreactivity in normal individuals. It is of particular interest because, on theoretical grounds, it could react with the PTH/PTHrP receptor and interfere with the biological effects of PTH(1–84).

Hypercalcemia acutely suppresses PTH secretion to a non-suppressible and detectable PTH concentration (9) while hypocalcemia stimulates PTH secretion to a maximum (9), both stimuli contributing to the sigmoidal relationship between Ca^{2+} and PTH concentrations (10). Ca^{2+} concentration also influences PTH secretion

qualitatively (2, 3, 11, 12). While suppressing PTH concentration, hypercalcemia favors C-PTH fragment secretion over that of PTH(1–84) (11), C-PTH fragments now accounting for 90% of circulating PTH immunoheterogeneity, and PTH(1–84) only 10%, causing a high C-PTH/PTH(1–84) ratio in the circulation (2, 3, 12). Hypocalcemia stimulates the secretion of PTH(1–84) more than that of C-PTH fragments (11), PTH(1–84) now accounting for 30% of circulating PTH immunoheterogeneity, and C-PTH fragments 70%, causing a low C-PTH/PTH(1–84) ratio in the circulation (2, 3, 12). This acute control of PTH secretion and of circulating PTH molecular forms is post-translational (13–15). PTH synthesis always proceeds maximally in the parathyroid glands, and the amount of PTH available for secretion is controlled by a Ca^{2+} -dependent degradation process (13–15) involving destruction of secretory granule content in lysosomes and possibly also within secretory granules (16–18). In rats, the predominance of one type of secretory granule over the other depends on Ca^{2+} concentration (17). C-PTH fragments are generated and secreted from these processes (19, 20). This type of regulation is rapid (13–15) and probably best explains the decrease in mean stimulated I-PTH observed by Bas *et al.* (1) after hypercalcemic clamp in normal rabbits. Similar results have been observed in cows (21), dogs (22) and humans (23). These modifications are of short duration and can be reversed by a hypocalcemic stimulation that is long enough (23). It is unlikely that other mechanisms are involved in this acute regulation of parathyroid function observed by Bas *et al.* (1) in view of the short time-course of their experimentation.

The significance of these quantitative and qualitative changes in parathyroid function and circulating PTH molecular forms may arise from two recent studies (24, 25) where human (h) PTH(7–84), and other synthetic C-PTH fragments, to a lesser extent, were found to have biological effects of their own and to antagonize some PTH(1–84) and PTH(1–34) biological effects. hPTH(7–84) was used in these studies as the only available representative of non-(1–84)PTH, with hPTH(39–84) and (53–84) as representatives of other circulating C-PTH fragments. hPTH(7–84) was

used alone or with the other synthetic C-PTH fragments, in a mixture, in an attempt to duplicate the *in vivo* situation. In both cases, hPTH(7–84) decreased Ca^{2+} concentration in thyroparathyroidectomized rats and antagonized the calcemic effect of PTH(1–84) (24, 25) or PTH(1–34) (25) in the same animals. The Ca^{2+} level observed during the combined infusion of PTH(1–84) and PTH(7–84) was the net result of addition of the positive influence of PTH(1–84) and of the negative influence of PTH(7–84). These results suggested that a high C-PTH/PTH(1–84) ratio in the circulation tended to decrease the PTH(1–84) calcemic action, while a low ratio would do the reverse, allowing more rapid and efficient control of calcium concentration in blood. PTH(7–84) was also found to reduce the phosphatemic and phosphaturic influence of PTH(1–84) (24, 25) and PTH(1–34) (25), and to bind to a C-PTH receptor and not to the PTH/PTHrP receptor in ROS 17/2.8 clonal cells, suggesting a mechanism of action through a receptor different from the PTH/PTHrP receptor (25). Since C-PTH receptors exist in large quantities on osteocytes (26), we postulated that the rapid anticalcemic effect of PTH(7–84) could involve the inhibition of osteocytic osteolysis and/or the stimulation of calcium/phosphate accretion into bone. More studies will be required to demonstrate that these results are applicable to endogenous non-(1–84)PTH and other C-PTH fragments, once the exact structure of non-(1–84)PTH is elucidated.

The influence of chronic hypercalcemia on parathyroid function and on circulating PTH molecular forms is more difficult to analyze because one must take into account phosphate and 1,25-dihydroxy-vitamin D ($1,25(\text{OH})_2\text{D}$) levels which also influence parathyroid function. This is best illustrated by the experimental model chosen by Bas *et al.* (1), where hypercalcemia was induced in rabbits by chronic renal failure and was accompanied by low circulating levels of $1,25(\text{OH})_2\text{D}$ and phosphate. One could also take as an example sarcoidosis where hypercalcemia would be present with high $1,25(\text{OH})_2\text{D}$ and phosphate levels (27).

First, one must ask if the influence of acute hypercalcemia on parathyroid function and PTH molecular forms is still present in chronic hypercalcemia. The answer is probably yes. We have demonstrated that patients with non-parathyroid hypercalcemia, while having a mean basal I-PTH level similar to that observed in normal individuals made acutely hypercalcemic, had a more elevated C-PTH level and a higher C-PTH/I-PTH ratio (28). We have also demonstrated, in various chronic experimental models associated with a need for higher PTH levels (secondary hyperparathyroidism, half parathyroidectomy) or lower PTH levels (treatment of secondary hyperparathyroidism, $1,25(\text{OH})_2\text{D}$ administration), further adaptation of the C-PTH/PTH(1–84) ratio to hypo- and

hypercalcemia compared with acute experiments. A chronic need for higher PTH levels was associated with a further decrease in the C-PTH/PTH(1–84) ratio at all Ca^{2+} concentrations while a chronic need for lower PTH levels was the reverse (29–31), indicating further adaptation of the C-PTH/PTH(1–84) ratio to chronic stimuli, possibly via changes in intracellular PTH degradation. These findings suggest that this type of control still exists with further adaptation in chronic experimental models.

Why was parathyroid function or at least stimulated I-PTH then unchanged with chronic hypercalcemia in the Bas *et al.* (1) study? This is probably best explained by the net result of several opposing influences exerted either at the transcriptional or post-transcriptional level by calcium, phosphate and $1,25(\text{OH})_2\text{D}$ concentrations, with a predominant effect of the latter. Chronic hypercalcemia slightly decreases preproPTH mRNA while hypocalcemia does the reverse (32–37). Chronic hypophosphatemia also decreases preproPTH mRNA while hyperphosphatemia does the reverse (38). Both calcium and phosphate concentrations act post-transcriptionally to stimulate or inhibit the production of proteins which bind to preproPTH mRNA to influence its stability and, thus, its degradation (38). Thus, one would expect preproPTH mRNA to be decreased in the Bas *et al.* (1) experiment based on Ca^{2+} and phosphate concentrations but, obviously, this is probably not the case because of the dominant influence of low $1,25(\text{OH})_2\text{D}$ level on PTH gene transcription (39, 40), preproPTH mRNA level (41) and PTH secretion (42). Several clinical situations can be used to illustrate the point. Treatment with $1,25(\text{OH})_2\text{D}$ in patients with renal failure decreases basal and stimulated I-PTH levels even if hyperphosphatemia is present (43). Stopping $1,25(\text{OH})_2\text{D}$ therapy in patients with renal failure increases PTH levels to high pretreatment values (44). In a rare case of sarcoidosis in an anephric patient with hypercalcemia, hyperphosphatemia, low PTH and high $1,25(\text{OH})_2\text{D}$ concentrations, treatment with cortisone led to decreased $1,25(\text{OH})_2\text{D}$ and higher PTH levels (45). Finally, in a patient with severe primary hyperparathyroidism and osteitis fibrosa cystica, $1,25(\text{OH})_2\text{D}$ concentration in serum was demonstrated to be low to start with in relation to the severe hypercalcemia (46). Treatment with i.v. bisphosphonate and vitamin D supplementation prior to surgery reduced Ca^{2+} concentration to a near normal level but with very high $1,25(\text{OH})_2\text{D}$ concentration and I-PTH and C-PTH levels half of what they were prior to treatment, even if the Ca^{2+} level had decreased (46). All this illustrates that the $1,25(\text{OH})_2\text{D}$ transcriptional influence on the PTH gene is probably dominant over the post-transcriptional role of calcium or phosphate concentration. In fact, in the model of Bas *et al.* (1), if the transcriptional influence of $1,25(\text{OH})_2\text{D}$ is sufficiently important, all other post-transcriptional and

post-translational controls could be present and effective, and the net result could still be a normal stimulated I-PTH level. Correction of the 1,25(OH)₂D level in rabbits with chronic renal failure to the value observed in normal rabbits through supplementation would probably have permitted better appreciation of other levels of PTH function regulation in this experimental model.

Overall, the paper of Bas *et al.* (1) illustrates the complexity of the acute and chronic regulation of parathyroid function *in vivo* and raises the question of the priority of a given stimulus over others in this regulation, leaving room for further experimentation.

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Received 7 August 2001

Accepted 9 November 2001