Parathyroid hormone regulation in normal and uremic rats

Reversibility of secondary hyperparathyroidism after experimental kidney transplantation

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INTRODUCTION

Uremia is associated with the development of often severe secondary hyperparathyroidism (sec. HPT). Therefore, understanding of the regulation of the parathyroid function is of significant importance for the nephrologists. Sec. HPT is associated with an increase in parathyroid hormone (PTH) gene expression, an increase in PTH synthesis and secretion and with proliferation of the parathyroid cells (7-11). At the molecular level the abnormal function of parathyroid glands is in chronic uremia currently related to disturbances of the calcium-sensing receptor (CaR), of the vitamin D receptor (VDR), and of the posttranscriptional regulation of PTH mRNA in the parathyroid cells (12-18). The stimuli for the development of sec. HPT of relevance to chronic renal failure (CRF) are hypocalcaemia, hyperphosphatemia, and low 1,25(OH)2D (7, 14, 19-23). Hyperparathyroidism early in uremia has important homeostatic functions by regulating the 1α-hydroxylase activity and phosphorus excretion. In more advanced uremia some degree of HPT is necessary for the maintenance of normal bone turnover. A further increase in the secretion of PTH will, however, lead to development of renal osteodystrophy with a high turnover bone disease (24-26) and affect the function of several organs (27-30). A successful renal transplantation may correct the abnormalities in mineral metabolism that lead to sec. HPT, although persistent high secretion of PTH may continue in some kidney transplanted patients (31-40). Therefore, the need is to control the sec. HPT at a desirable level and to avoid the development of non-suppressible and non-reversible changes in the parathyroid glands. The clinical evaluation of sec. HPT has proven not to be an easy task. The circulating level of PTH is a poor indicator of renal osteodystrophy (41-44). Biopsy of the glands is not possible. Sufficiently sensitive techniques for evaluation of the glanular mass are not available. The inverse relationship between plasma Ca2+ and PTH and on the interpretation of the Ca2+/PTH relationship is not possible. Sufficiently sensitive techniques for evaluation of the glanular mass are not available. The inverse relationship between plasma Ca2+ and PTH has been examined in uremic and kidney transplanted patients by several groups (45-48). Most of the interpretation using the Ca2+/PTH relationship as a measure for the parathyroid mass and parathyroid gland function are, however, based on unproven assumptions and should therefore be taken with some precaution (49).

In the present investigations the parathyroid function was examined in rats where sec. HPT was induced by partial nephrectomy (PNX) and by different dietary manipulations (2, 3, 5). Thus, sec. HPT was examined in experimental models of relevance for the clinical situation. An unique model of reversible uremia was created by performing an experimental kidney transplantation in previously long-term uremic rats (1, 2, 5). In this model chronic uremia for up to 20 weeks was reversed by the isogenic kidney transplantation, permitting examination of the reversibility of sec. HPT without any interference from immunosuppressive treatment (1). The parathyroid glands were removed by microsurgery for examination of CaR and VDR gene expressions in uremia and after kidney transplantation (5). Furthermore, a model of parathyroid hyperplasia was created by implantation of several normal or uremic isogenic parathyroid glands into one rat in order to establish the impact of a significant increase of the parathyroid glandular mass on the levels of plasma Ca2+ and PTH and on the interpretation of the Ca2+/PTH relationship (2).

The parathyroids are not controlled by a superior “hypothalamic-pituitary axis” and are therefore likely to use autocrine/paracrine regulatory mechanisms. Autoreceptor effects of some hormones have been documented (50-52). In the present investigation it was examined whether PTH might exert a feedback regulatory effect on its own secretion from the parathyroids. The existence of positive feedback will allow for explanation of several phenomena in the parathyroid glands and add further to the understanding of the Ca2+/PTH relationship (4, 6).

The aim of the present thesis was:

1. to create experimental models, that could improve our understanding of the Ca2+/PTH relationship in normal and uremic animals,
2. to study the possible reversibility of secondary hyperparathyroidism, induced by severe uremia, after reversal of uremia and normalization of the kidney function by a successful, experimental, isogenic kidney transplantation,
3. to study the relationship between parathyroid mass and PTH secretion in relation to Ca2+,
4. to study the relationship at the molecular level between PTH secretion and the parathyroid CaR and VDR in uremia and after normalization of the kidney function, and
5. finally, to examine whether a feedback autoregulation exists within the parathyroid glands between PTH/PTHrP receptor ligands and the secretion of PTH, as such a feedback mechanism might help further to understand the relationship between Ca2+ and PTH.

METHODS

INDUCTION OF UREMIA (CRF) AND SECONDARY HYPERPARATHYROIDISM (SEC. HPT)

Inbred Dark Aguti (DA) or Lewis rats were used.

- CRF: was induced by one step 5/6 nephrectomy, as previously described in details (1, 2).
- Sec. HPT: was induced in 2 models (2):
  - Model A: CRF-rats were given a standard diet containing 0.9% Ca, 0.7% phosphorus, and 1000 IU vit. D/kg in order to induce moderate sec. HPT.
  - Model B: CRF-rats were given a high phosphorus diet containing 0.9% Ca, 1.5% phosphorus, and 1000 IU vit. D/kg in order to induce severe sec. HPT.

A MODEL OF REVERSIBLE UREMIA

After a period of up to 20 weeks of chronic uremia, the uremia was reversed by an isogenic kidney transplantation. The kidney was transplanted into the left orthopic side with end-to-end anastomoses of the blood vessels and the ureter. This technique has previously been described in details (1). A major technical difficulty was the extreme fragility of the recipients vessels, that had not been in function during the long period of uremia and were left atriopic without the usual elasticity and therefore were considerable smaller than the donor vessels. The donor rats were heparinized prior to the nephrectomy. No perfusion of the graft was performed prior to
transplantation. The warm ischemia time was between 40-50 minutes and the new transplanted kidney produced urine immediately after the vessel anastomoses had been completed. There were no signs of rejection at histological examination (unpublished data). The great advantage of this difficult and time consuming model is first of all, of course, that this transplantation is not associated with any kind of rejection and that it does not require any kind of medical treatment, i.e. no immunosuppressive and no antihypertensive treatment. One week after kidney transplantation the kidney function was normalized and plasma-calcium, -phosphorus and -magnesium became normal (1, 2, 5).

THE SECRETORY RESPONSE OF PTH TO HYPO- AND HYPERCALCEMIA
Parathyroid function was examined as the secretory response of PTH to hypocalcemia and hypercalcaemia. Hypocalcemia was induced by an infusion of 30 mM EGTA (ethylene-bis(oxyethylene-nitri-lo) tetracetic acid, Sigma, USA), 3 ml/hour, by a Braun minipump, via a catheter inserted in a femoral vein. Samples for determination of plasma-Ca²⁺ and plasma-PTH were obtained at 0, 5, 10, 20, 30, 40, and 50 minutes from a corresponding catheter in the femoral artery (2, 3). Hypercalcaemia was induced by an infusion of 25 mM CaSandoz, 3 ml/hour via a catheter inserted in the femoral vein. Samples for determination of plasma-Ca²⁺ and plasma-PTH were obtained at the times indicated above (2).

PARATHYROIDECTOMY
Parathyroidectomy was performed as a selective removal of the two parathyroid glands and was ensured by a drop in plasma PTH to undetectable levels within 60 min. (2).

MODELS OF PARATHYROID HYPERPLASIA
A model of pure parathyroid hyperplasia was introduced by an isogenic implantation of 20 normal parathyroid glands into one rat, 60 min. after PTX of the its own 2 parathyroid glands (2).

A transient period of hypercalcemia occurred initially after the increase of the parathyroid mass. Within 2 weeks, however, plasma calcium returned to normal levels and remained normal for the following 6 weeks of observation (2). PTH levels were normal from the third day after implantation of the 20 parathyroid glands and remained normal for the following 6 weeks (2).

In a model of uremic hyperplasia, 8 parathyroid glands from hyperparathyroid uremic rats on a standard diet were implanted into the neck muscles of normal recipient rats, after PTX of their own parathyroid glands. PTH levels remained within the normal range, despite the implantation of uremic hyperparathyroid glands. In parallel with the 20 glands model a transient hypercalcemia was observed in this model of uremic hyperplasia.

In a control model on the viability of the implanted glands, 8 normal parathyroid glands were implanted into uremic hyperparathyroid rats that were kept on a high phosphorus diet, after PTX of their own parathyroid glands. In these uremic rats with new-im- planted "normal" glands severe hyperparathyroidism re-developed on the third day. Thus, the viability of the implanted parathyroid glands was confirmed by the rapid development of hyperparathyroidism in this control model (2).

DETERMINATION OF CAR, VDR AND PTH/PTHrP RECEPTOR mRNA
The parathyroid glands were ultrasonicated to allow for complete cell rupture. Then total RNA was extracted and quantified by spectrophotometry. VDR vs Actin, CaR vs Actin and PTH/PTHrP receptor mRNA vs Actin were amplified separately using the Access-RT-PCR System (Promega, Madison WI). Each sense primer was marked with 6FAM fluorocrome. The PCR was stopped when the reactions were in the linear range. DNA amplifications were processed using a Gene Scan Analyzer, Abi Prism 310 (Perkin Elmer, CA, USA). Data was analyzed with specific software Gene Scan version 3.1/1998 (Perkin Elmer) (5, 6).

PLASMA MEASUREMENTS
Plasma-PTH was measured by a rat PTH (IRMA) assay provided by Nichols Institute, Ca, USA or Immunotopic, Ca, USA (the same assy). The intra-assay coefficient of variation was in our lab. 4%, and the inter-assay coefficient of variation was 35% for a PTH level of 7 pg/ml, 5% for PTH of 40 pg/ml, and 5% for PTH of 260 pg/ml. The samples for comparison of PTH suppressibility by high calcium were run in the same assay. No cross-reactivity with C-terminal PTH fragments or N-terminal PTHrP was found in our lab. (2, 4).

In the studies in ref. (1) PTH was measured by an older competitive radioimmunoassay for PTH, using a chicken antibody raised against synthetic human PTH 1-34 and cross-reacting with rat PTH (INS-PTH assay from Nichols Institute, Ca, USA). The intra-assay and the inter-assay coefficients of variation were 5%. 1,25(OH)₂D₃ was measured by a competitive protein assay using calf thymus as the source of binding protein. 1,25(OH)₂D₃ was extracted from plasma as described by Reinhardt et al. (53). The intra-assay coefficient of variation was 4%, the inter-assay coefficient of variation was 14% for a 1,25(OH)₂D₃ level of 35 pg/ml, as measured in our lab. (2). P-creatinine, urea, phosphorus, magnesium and total Ca were measured by an ETACHEM 250 Analyzer, KODAK. Plasma Ca²⁺ was measured at actual pH by a calcium selective electrode from Radiometer, Copenhagen, Denmark.

STATISTICS
The results were expressed as mean ± SEM. The Mann-Whitney test was used for the comparison between groups or a t-test was used for groups of data with equal SD. Differences between more than two means were evaluated by ANOVA followed by the Duncan test. P <0.05 was considered significant.

THE CALCIUM/PTH RELATIONSHIP
IONIZED CALCIUM (Ca²⁺) AND THE CALCIUM-SENSING RECEPTOR (CaR)
Plasma Ca²⁺ is the major determinant of the secretion of PTH from the parathyroid glands. The cloning and characterisation of a calcium-sensing receptor (CaR) in the plasma membrane of parathyroid cells brought important and fascinating new information on the mechanisms by which Ca²⁺ and other ions control the parathyroid function (54). The detailed mechanism behind the complexity of the calcium regulated PTH secretion is, however, not yet well understood (54, 55). The parathyroid glands express abundant CaR mRNA and protein (15, 54, 56). The CaR belongs to the superfamily of G-protein-coupled receptors (54, 57). CaR agonists activate phospholipase C (PLC), phospholipase A₂ (PLA₂) and phospholipase D (PLD) in the parathyroid cell (58). Activation of PLA₂ and PLD involve protein kinase C (PKC) dependent pathways that are activated by the CaR, presumably via PLC (58, 59). High extracellular Ca²⁺ elicits a transient rise in intracellular Ca²⁺, probably as a result of activation of PLC and of the resulting inositol-tri-phosphate (IP₃)-mediated release of Ca²⁺ from the intracellular stores (58). The high extracellular Ca²⁺ likewise induces a sustained increase in intracellular Ca²⁺ through incompletely defined Ca²⁺ influx pathways (60). PTH secretion is inhibited by the elevated intracellular Ca²⁺ (61, 62). Several lines of evidence support the role of the CaR as the key mediator of the inhibitory action of elevated extracellular Ca²⁺ on secretion of PTH. In patients with familial hypocalciuric hypercalcaemia (FHH), who are heterozygous for inactivating mutations of the CaR gene, a shift to the right has been shown in the relationship between calcium and the inhibition of PTH secretion (63, 64). Patients and mice that are homozygous for such mutations have a more severe impairment of the high calcium induced suppression of PTH secretion, as a documentation for the assumption that calcium "resistance" of the parathyroids is inversely related to the number of
functioning CaR's (57, 65). Furthermore, a reduction of CaR expression in cultured parathyroid cells is associated with a loss of inhibition of PTH secretion by high calcium (66, 67).

The Ca²⁺/PTH relationship and some factors that may affect this relationship are evaluated in the following. The Ca²⁺/PTH relationship during acute changes in Ca²⁺ is described by a sigmoidal Ca²⁺/PTH curve (45, 60, 68). Focus will be on the complexity of this Ca²⁺/PTH relationship. This is demonstrated by results from our lab. (2, 3) and by data the literature with specific attention to the significance of several the components of the Ca²⁺/PTH curve.

THE CA²⁺/PTH CURVE
The Ca²⁺/PTH curve is shown in Figure 1. The linear part of the curve is very steep. This means that just a small decline in Ca²⁺ below normal levels will result in a dramatic increase of the PTH secretion. The maximal secretory rate (the upper part of the curve) is accordingly reached in just a mild degree of hypocalcemia. Induction of even mild hypercalcemia will, in contrast, result in a modest decline of PTH secretion, as indicated at the lower part of the curve. It is, however, still questioned whether PTH secretion can be totally suppressed, even at very high Ca²⁺ concentrations (60, 68).

Several factors are involved in the complexity of the Ca²⁺/PTH relationship, such as intraglandular/intraglandular degradation, recruitment of parathyroid cells in an active state, autocrine/paracrine factors, hysteresis, rate-dependent control of the PTH response, the transient nature of the initial increase in the PTH response to a reduction of Ca²⁺ and several intraglandular factors.

INTRACELLULAR DEGRADATION OF PTH
Habner et al. (69) showed that the intracellular degradation of newly synthesized PTH was regulated by the extracellular Ca²⁺ concentration. When bovine glands were incubated in a hypocalcemic medium, almost all of the newly synthesized PTH was secreted as intact PTH. When exposed to a hypercalcemic medium most of the newly synthesized PTH was degraded in the glands (69). In accordance with this observation, Mayer et al. (70) demonstrated in vivo that PTH secreted into the parathyroid venous blood of calves mainly consisted of hormonal fragments, when hypocalcemia was induced. Thus, the parathyroid glands control the amount of hormone available for secretion by regulating the fraction of PTH that is either exocytosed or degraded. The acute regulation of the intracellular PTH degradation during induction of hyper- or hypocalcemia has, however, not yet been established in details.

RECRUITMENT OF PARATHYROID CELLS
Another intraglandular regulatory mechanism of possible importance for the secretion of PTH is the recruitment of parathyroid cells, as shown in studies from Ritchie et al. (71), who demonstrated that the parathyroid cells may cycle between active and inactive secretory states. As such, the secretory response of PTH to changes in Ca²⁺ may be altered by the fraction of parathyroid cells in an active secretory state (72, 73). The possible influence of the number of cells in an active secretory state on the acute response of PTH to fluctuations in Ca²⁺ has, however, not yet been established.

AUTOCRINE/PARACRINE REGULATION OF PTH SECRETION
An autocrine/paracrine regulation of PTH secretion might have a significant further effect on the Ca²⁺/PTH relationship. PTH, Chromogranin A (CgA), Chromogranin A related peptides and Endothelin-1 have all been suggested as factors that might affect the PTH secretory response. We have to the list of potential autocrine/paracrine factors added PTH/PTHrP receptor targeting molecules, such as N-terminal PTHrP and PTH itself (4, 6), as discussed on page 195.

HYSTERESIS
Hysteresis describes the phenomenon that the parathyroid cells are sensing the direction of changes of Ca²⁺ (74, 75). The levels of PTH are higher during induction of hypocalcemia, than at the same level of Ca²⁺, when the Ca²⁺ levels are raised again, resulting in two different curves (74, 75). The question, therefore, is which of the two curves - obtained during induction of hypocalcemia or during recovery from hypocalcemia - should be chosen as the one, which best is representing the parathyroid function?

Felsenfeld et al. (76) performed a careful study on the Ca²⁺/PTH relationship in 19 hemodialysis patients. First hypocalcemia was induced by a calcium-free dialysate and then recovery from hypocalcemia was induced by changing the Ca²⁺ concentration of the dialysate to 3.5 mEq/l. Two different curves were obtained for each single patient, one curve during induction of hypocalcemia and another one during recovery from hypocalcemia. These curves clearly demonstrated the phenomenon of hysteresis and showed that the set-points for calcium derived from the individual Ca²⁺/PTH curves were higher in all patients during induction of hypocalcemia, than during recovery from hypocalcemia. Interestingly, when the patients were divided according to their basal Ca²⁺ concentrations, then the Ca²⁺/PTH relationship showed much greater variation during induction of hypocalcemia than during recovery from hypocalcemia. Therefore, if the differences in the secretory responses of PTH do not result from different rates of Ca²⁺ reduction between the groups, then the conclusion must be that investigations on the Ca²⁺/PTH relationship should start at basal Ca²⁺ level. This principle was followed in the present studies.

RATE-DEPENDENT CONTROL OF THE PTH RESPONSE
Rate-dependent control of the PTH response indicates that a rapid decrement of Ca²⁺ results in a higher PTH response than the response obtained during a more slow decrement of Ca²⁺, even when reaching at the same level of Ca²⁺. Thus, a more rapid change in the rate of Ca²⁺ is associated with a higher shift upward and to the right of the relationship between PTH and Ca²⁺ (77, 78). It is therefore a question whether it is possible to determine the sensitivity of the parathyroid glands to Ca²⁺ by making Ca²⁺/PTH curves and whether from such curves the set-point for calcium can be estimated. The answer to this question is very important for the nephrologists in order to obtain a better knowledge of the mechanisms that are involved in the secondary hyperparathyroidism of uremia.

Figure 1. The Ca²⁺/PTH relationship in normal rats. Acute hypocalcemia was induced by an EGTA infusion and acute hypercalcemia by a Ca²⁺ infusion. N=6. Mean ± SEM.
and in order to obtain a better evaluation of the results from treatment with vitamin D analogs. The results of several clinical and experimental investigations are conflicting (79-84). An abnormal Ca\(^2+\) set-point in uremic parathyroid glands was shown in an in vitro investigation although a wider range of set-points appeared (85).

Studies in vivo are complicated to evaluate due to the different responses to induction of hypocalcemia and hypercalcemia which exist between patients (86). Only a few studies provide data on the differences in time required to obtain a certain level of hypo- and hypercalcemia. Kwan et al. (87) showed in patients on hemodialysis that the duration required to increase the Ca\(^2+\) concentration by 0.25 mmol/l from basal levels varied from 30-115 minutes. Similar great time differences from 55-155 minutes were found when Ca\(^2+\) was decreased by 0.25 mmol/l. It is obvious that the rate of induction of hypercalcemia and/or hypocalcemia have been dramatically different between these patients. Thus, according to the rate-dependency of the PTH response to Ca\(^2+\), the Ca\(^2+\)/PTH curves of these patients were obtained during significantly different circumstances and Kwan et al. (87) subsequently abstained from any estimation of the Ca\(^2+\) set-points. Mc Carron et al. (48) examined the parathyroid function in normal subjects and in kidney transplanted patients with persistent HPT and hypercalcemia. The authors noted significantly different decreases in Ca\(^2+\) during a two hour of a fixed dose of EDTA infused in the two groups (0.45 versus 0.96 meq/l).

Thus, disturbances of the Ca\(^2+\) homeostasis might have resulted in different rates of change in plasma-Ca\(^2+\) in response to EDTA or calcium infusions. Most other studies describe the Ca\(^2+\)/PTH relationship by a 2-dimensional model, which is omitting the time factor (49). Therefore, when considering the present knowledge on the Ca\(^2+\)/PTH relationship, most of these interpretations must be taken with some precaution. At present we do, therefore, unfortunately not have any useful model for the evaluation of the Ca\(^2+\) sensitivity of the parathyroid glands in uremic patients.

**WHAT LEVEL OF PTH REPRESENTS THE MAXIMAL SECRETORY RESPONSE AND WHAT DOES IT MEAN?**

As previously demonstrated (77, 78) the maximal response of PTH secretion to hypocalcemia is dependent upon the rate of reducing the Ca\(^2+\) levels. It is, therefore, a question whether in vivo studies the secretion of PTH, induced by hypocalcemia, always is performed at the "maximal" rate and as such will result in a maximal response. The transient nature of PTH secretion during induction of hypocalcemia adds difficulties to the interpretation of the maximum. During a severe and rapid decrease of Ca\(^2+\) an initial rapid increase of the PTH levels is seen, which soon declines to a lower level, despite a continuous fall in Ca\(^2+\). The PTH levels remain, however, considerably higher than before stimulation. This has been shown in patients (88) and in the rat (4).

What level should be considered as the maximum? To answer this question we need to understand the cause of the transient elevation of PTH. This is not known as yet. Some investigators will interpret the elevation as due to emptying the stores of preformed PTH (88). Thus, the subsequent levels of PTH secretion should express the ongoing biosynthesis of the hormone. This is an as yet unproved assumption, which is in contrast to the accumulating knowledge on the complexity of the regulation of PTH secretion (4). Thus, our results, which showed a dramatic, immediate and sustained enhancement of the low Ca\(^2+\) induced PTH secretion by PTHrP, are in contrast to the assumption of an initial emptying of all stored and preformed PTH during acute induction of hypocalcemia (4). Furthermore, it has been assumed that the "maximal" PTH level in response to hypocalcemia reflects the "maximal secretory capacity" of the parathyroid glands and as such should be an indicator of the mass of the parathyroids (46, 82, 89).

Our results (4, 6) from in vivo and in vitro experiments demonstrated for the first time that PTHrP significantly enhanced the low Ca\(^2+\) stimulated PTH secretion. Furthermore they clearly showed that the level of PTH that previously had been considered as an expression of the "maximal secretory capacity" of the parathyroid cells in fact was not the maximum, but that "the maximal" PTH secretion could be increased even further, by several fold.

**WHAT IS THE MINIMAL SECRETION OF PTH?**

It is generally accepted, that PTH secretion can not be totally suppressed even at very high concentrations of Ca\(^2+\) (90). Not all evidence is, however, in agreement with this point of view.

We have to question, whether the non-suppressible secretion of PTH 1-84 exists at all in normal subjects. The observation, that PTH secretion can not be completely suppressed by Ca\(^2+\) was obtained in vivo studies at a time, when the available assays were co-measuring PTH fragments (91) and later confirmed in in vitro studies on cultured parathyroid cells (85). The secretion of PTH fragments in vivo studies might be the result of a massive intraglandular degradation of PTH during the condition of hypercalcemia (92).

Furthermore, there might be non-parathyroid sources of some of the circulating PTH, such as hypothalamus or thymus. The PTH gene is expressed in the hypothalamus (93). Recently, it has been shown that transgenic mice (deficient in the Gm2-gene, a master regulatory gene of parathyroid gland development) that are lacking the parathyroid glands, had circulating PTH secreted from the thymus (94). In in vitro studies it has been well documented that cultured parathyroid cells loose their Ca\(^2+\) sensitivity very fast (67), and that a high calcium containing medium only can suppress PTH secretion by approximately 50-75% of the maximal low Ca\(^2+\) stimulated secretion (95). Brown et al. (67) reported, that the Ca\(^2+\) mRNA declined by 70% during the first 4 hours in a culture of bovine parathyroid cells and remained very low for the following 24 hours of culture. Thus, loss of calcium responsiveness in cultured parathyroid cells and thus the non-suppressibility might be due to a dramatic drop in the expression of the Ca\(^2+\).

By the use of assays that measure the so-called intact PTH in normal experimental animals the minimal secretion of PTH during conditions of hypercalcemia in vivo is remarkably low and probably below detection limit of the assays. Thus, in an in vivo study on normal dogs Cloutier et al. (96) suppressed intact PTH to 0.10±0.06 pmol/l. Of course the sensitivity of the intact PTH assay has to be considered even at very high concentrations of Ca\(^2+\). Finally, the late phase of the metabolism of PTH (or PTH fragments co-measured) might be delayed, and as such the very low levels of PTH measured in hypercalcemia might be due to the expression of the very slow metabolism and not due to a non-suppressible secretion of PTH. In support of this concept Singer et al. (99) found that the disappearance rate of bovine PTH in dogs was multiexponential with an initial T\(_1/2\) of 4-8 minutes and a terminal T component of 54-99 minutes. In uremia the metabolism of PTH might be further delayed, as postulated by Schmitt et al. (100). The observation of pulsatile PTH secretion in hypercalcemia by Schmitt et al. (100, 101) is in favor of the existence of a non-suppressible PTH secretion, although also in this study cosecretion of C-terminal PTH fragments might have played a role.

**THE EFFECT OF PHOSPHORUS ON THE MINIMAL SECRETION OF PTH**

Phosphorus has a direct stimulatory effect on PTH secretion (22, 95, 102, 103). Phosphorus retention might be associated with impaired suppressibility of PTH secretion in uremia (45, 104). We have (2) shown in hyperphosphatemic uremic rats that even severe hypercalcemia was not able to suppress PTH secretion to the same extent as that of normal rats or that of uremic normophosphatemic rats. This malfunction was normalized after reversal of uremia by a kidney transplantation (2) (Figure 2). These results underline the importance of phosphorus in the abnormal suppressibility of PTH secre-
2.00

Uremia-P  TX  Controls

Figure 2. The Ca2+/PTH relationship during acute inductions of hypocalcemia and hypercalcemia in uremic rats kept on high P diet (Uremia-P), kidney transplanted (TX), and in normal control rats (Controls). The PTH secretory response to hypocalcemia was significantly higher in uremic rats (p<0.001) than in kidney transplanted and normal control rats. The PTH secretion was suppressed to same extend in kidney transplanted rats and normal control rats, while uremic hyperphosphatemic rats had a significantly higher (p<0.05) PTH secretion at high Ca2+. Due to the complexity of the Ca2+/PTH relationship, such as rate dependency and ability of the parathyroids to sense the direction of calcium changes, the set-point for calcium in the parathyroids can not be determined from the present curves. For details see reference (2). N=6, mean ± SEM.

planted, that PTH secretion can be suppressed to the same low level as observed in normal rats (2). Thus, the complexity of the regulation of PTH secretion during hypercalcemia provides the possibility for several different explanations for the previous findings of abnormal levels of non-suppressible PTH. It is concluded, that the Ca2+/PTH concept, beside being influenced by the rate and/or direction of changing the Ca2+ concentration also might be the result of an interaction between Ca2+ and several autocrine or paracrine factors and of changes in the metabolism of PTH. It is still an unresolved question, how the regulation of these factors interplay under physiological and pathophysiological circumstances and how they may affect the curves that are representing the detailed Ca2+/PTH relationship.

PTH AND THE MINUTE-TO-MINUTE CALCIUM HOMEOSTASIS

Calcium has important extracellular as well as intracellular functions. Its extracellular functions include its role in blood clotting, maintenance of plasma membrane integrity and intercellular adhesion. Extracellular calcium provides a constant supply for the exchange of calcium within the large reservoir of calcium - the skeleton. The skeleton acts as a rigid framework that facilitates body movement, intake and digestion of food, and which protects soft tissues. The skeleton represents the largest compartment of total body calcium, containing more than 99%.

Intracellular calcium is an important intracellular second messenger regulating multiple cellular functions, such as metabolism, motility, secretion and proliferation (106-108). It is a cofactor for several intracellular enzymes, e.g. mitochondrial dehydrogenases, various phospholipases and proteases (60, 109, 110). The free extracellular Ca2+ concentration is maintained within a narrow range (111). A major emphasis has traditionally been placed on the role of the calciotropic hormones i.e. PTH, 1,25(OH)2D and calcitonin in maintaining the extracellular calcium homeostasis (60, 112). The traditional and well-established model of the overall calcium homeostasis has two key components. The first comprises of several distinct cell types that sense changes in the extracellular Ca2+ leading to appropriate changes in the secretion of the calciotropic hormones. The second key component is the effector systems, specialized calcium-translocating cells of the kidneys, bones and intestine, that respond to the calciotropic hormones with changes in the transport of mineral ions in order to restore the extracellular Ca2+concentration to normal (60).

RESPONSE TO HYPOCALCEMIA - THE TRADITIONAL MODEL ON CALCIUM HOMEOSTASIS

The current model on calcium ion homeostasis provides the following explanation for the subsequent normalization of plasma calcium after a short induction of hypocalcemia (60, 113, 114). Even a slight reduction in the extracellular Ca2+concentration elicits a prompt increase in the rate of PTH secretion. The renal responses to the increased plasma PTH levels of relevance to the calcium ion metabolism include phosphaturia, enhanced distal tubular reabsorption of Ca2+ and increased generation of the active metabolite of vitamin D, 1,25(OH)2D from 25(OH)D by the stimulation of the 1α-hydroxylase in the proximal convoluted tubules. The increased levels of 1,25(OH)2D stimulate the intestinal absorption of phosphate and calcium. PTH and 1,25(OH)2D promote net release of phosphate and calcium from bone. The increased flux of Ca2+ into the extracellular fluid, coupled with renal retention of Ca2+ restore circulating Ca2+ levels toward normal, and thereby inhibit PTH secretion and close the negative feedback loop. The excess of phosphate mobilized from intestine and bones is excreted into the urine. In this traditional model of calcium ion homeostasis the emphasis is on the key role of PTH, as the extracellular first messenger.
THE MINUTE-TO-MINUTE REGULATION OF PLASMA Ca²⁺ DOES NOT DEPEND UPON THE CALCIOTROPIC HORMONES, PTH AND 1,25(OH)₂D³

Additional regulatory mechanisms may, however, be involved in the acute and very rapid recovery of plasma calcium that is seen after an acute induction of hypocalcemia. This was demonstrated in the following experiments from our group (3). Parathyroidectomy was performed in the rat. Sixty minutes or 24 hours later plasma Ca²⁺ was reduced by a brief infusion of EGTA. Already 10 minutes after discontinuation of the EGTA infusion plasma Ca²⁺ increased significantly and reached the level of the vehicle-infused rats within another 2 hours. The same very rapid recovery from an EGTA-induced hypocalcemia was observed in rats that were both acutely PTX and nephrectomized (PNX) (3). Thus, a rapid and within minute recovery of plasma Ca²⁺ took place immediately after termination of the induction of hypocalcemia, despite no PTH present in the circulation and despite the elimination of the renal handling of calcium (3). These results suggest that PTH might not be of significant importance for maintaining the extremely stable extracellular calcium balance and point toward the existence of other factors or mechanisms involved in the rapid minute-to-minute regulation of plasma Ca²⁺ (3).PTH is, however, setting the level of plasma Ca²⁺, which is maintained by this complex homeostatic system (115). In this concept it was shown, that rats which were aparathyroid for 24 hours became hypocalcemic – as expected – and that plasma Ca²⁺ levels recovered after a further short reduction of plasma Ca²⁺ by an EGTA infusion, but only to the new basal level of the hypocalcemic aparathyroid rats (3). When hypercalcemia was induced in rats by a continuous infusion of exogenous PTH, plasma Ca²⁺ levels recovered after a brief induction of hypocalcemia to the new hypercalcemic level (116). This is in agreement with the previous results of Coop et al. (117-119), who in normal and thyroparathyroidectomized dogs measured total calcium after an EGTA infusion and observed that calcium increased to a level set by the PTH status. Similarly Wang from our group (120) demonstrated that 1,25(OH)₂D₃ did not affect the rapid calcemic recovery from EGTA induced hypocalcemia, but that the effect of 1,25(OH)₂D₃ was on the long-term plasma calcium homeostasis in the rat. Plasma Ca²⁺ levels recovered after a short induction of hypocalcemia at the same rate in vitamin D depleted rats as in rats treated with different doses of 1,25(OH)₂D₃. The final levels of plasma Ca²⁺ obtained were, however, set by 1,25(OH)₂D₃ in a dose-related manner (120).

THE CONCEPT OF A "SET POINT" FOR Ca²⁺ IN THE PARATHYROID, THE KIDNEYS AND BONE

The conceptual framework on how plasma calcium is held constant is outlined by Kurokawa (115). He described a concept, which combined the "set point" for the calcium flux between extracellular fluid volume (ECF) and bone, and the "set point" for renal tubular calcium reabsorption in relation to the "set point" for the calcium regulated PTH secretion from the parathyroids (115). The interplay between PTH and 1,25(OH)₂D₃ will set the ECF calcium effectively in its own regulation through systemic and local receptor-mediated actions of Ca²⁺ on the kidney and other tissues related to the mineral ion homeostatic system (130, 131). Thus, the capacity of ion-translocating cells within the mineral ion homeostatic system that recognize and respond to changes in their local ionic environment may play a more fundamental role in the ion homeostasis than previously recognized. Evidence in strong support of this postulate is coming from an "experiment in nature". Individuals with familiar hypercalcemic hypocalcuria (FHH) represent inactivating mutations in the CaR. The CaR is located in several regions of the kidney, including the distal nephron. The clinical feature of FHH is a reduction of the fractional renal clearance of Ca²⁺, independent of PTH and despite concomitant hypercalcemia (132, 133). Thus, calcium sensing (134) and calcium handling might be regulated locally in the kidney and hereby influence the calcium homeostasis supporting the concept that effector tissues in the mineral ion homeostatic system recognize and respond to changes in plasma Ca²⁺ (3). This new concept creates a possibility for the existence of a calcium sensing system at the quiescent bone surface, where the labile pool of calcium ions has been shown (135). This labile pool may function as a short-term buffer, taking up or releasing calcium to correct for changes of Ca²⁺ in the ECF.

CAR AND BONE

Although a calcium sensing mechanism at the quiescent bone surface will represent an attractive model for the rapid minute-to-minute regulation of plasma Ca²⁺, we have to conclude, that despite evidence for an important role of the CaR in recognizing and responding to changes in plasma Ca²⁺ at the level of the parathyroids and kidneys no such evidence has yet been provided at the level of bone. The existence of a calcium sensor in the skeleton is largely supported by indirect evidence. As such, it is suggested that the extracellular Ca²⁺ concentration in the bone micro-environment might play a physiological role in bone remodeling (136, 137). The CaR has variably been reported to be expressed in bone-like cells, as the osteoblast-like cell lines (138-141), the stromal cell line (142) and the osteoclast precursor cells (140). Definitive evidence for a physiological role of the CaR in bone remodeling is, however, still lacking. Recent studies on the phenotype of CaR knockout mice (143) failed to find support for a CaR mediated regulation of osteoclast activity and osteoblast recruitment. Surprisingly, rickets was the predominant skeletal abnormality in these animals (143). As such, the CaR might have a role in the mineralization of bone. Whether this putative novel role of the CaR is related to calcium sensing at the quiescent bone surface is not known, as the mechanisms remain to be elucidated. Although the CaR has been shown in bone-like cells, there is at present no solid evidence for expression of the re
receptor in mineralized bone containing mature osteoblasts and osteocytes (144). Furthermore, studies using a CaR agonist or an antagonist did not support a role of an eventual CaR in bone for the regulation of plasma Ca$^{2+}$ (145-147). As such, no effect of the agonist was observed on plasma Ca$^{2+}$ in TPTX animals (145). However, the detailed studies on the recovery of plasma Ca$^{2+}$ from EGTA induced hypocalcemia together with administration of CaR agonists or antagonists have still to be performed.

**ADDITIONAL CA$^{2+}$SENSORS IN BONE**

Recent investigations in bone cells have provided evidence for the existence of an additional Ca$^{2+}$ sensor (141), that is different from the CaR, which originally was cloned by Brown et al. (54). Future studies may unveil whether this new Ca$^{2+}$ sensor mediates some or more of the known effects of Ca$^{2+}$ on bone cells (141, 148) and whether this sensor participates in the regulation of fluxes of calcium between bone and ECF. The identity of a putative novel cation receptor remains unknown.

**THE POSSIBLE EXISTENCE OF A NEW FACTOR IN THE RAPID REGULATION OF PLASMA CA$^{2+}$**

The mechanism responsible for the rapid minute-to-minute regulation of plasma Ca$^{2+}$ is still unsolved (3). The possibility of a circulating factor or hormone, that is participating in the rapid regulation of the calcium flux between bone and ECF still have to be considered. An old observation, which showed that the calcium-buffering ability is a function of the intact organism and could not be reproduced in an isolated perfused limb, supports such a possibility (149). Calcitonin is a fast acting hormone and Wang from our group have shown that acute thyroidectomy in the rat resulted in an acute increase of plasma Ca$^{2+}$ (150). Thus, we speculated whether acute suppression of the calcitonin secretion by EGTA induced hypocalcemia could be responsible for the rapid recovery of calcium (3). We did, however, fail to prove this hypothesis, as the calcium recovery was preserved in thyroparathyroidectomized rats that were infused with exogenous calcitonin (150). Therefore, our results (3, 116, 120, 150) point towards the existence of factor other than PTH, and other than calcitriol or calcitonin, which is involved in the rapid minute-to-minute calcium homeostasis. It is therefore of interest, that still new humoral factors or hormones, that are participating in the mineral metabolism, are being characterized (151). Such a new factor is fibroblast growth factor-23, a new phosphaturic factor, that is isolated from tumors of patients with tumor-induced osteomalacia and which is a candidate for a phosphate regulating-hormone, phosphatonin (151-155). The FGF-23 gene is expressed in human heart, liver, thyroids, parathyroids, small intestine, testes, skeletal muscles and fetal chondrocytes. Serum levels of FGF-23 are measurable in healthy adults and enhanced in patients with tumor-induced osteomalacia (156). The circulating levels are possibly regulated by proteolytic cleavage by a metallo-endopeptidase PHEX (95). Excess of FGF-23 inhibits the renal phosphate reabsorption by regulating the expression of the sodium/phosphate co-transporter in the proximal nephron (157). The characterization of more phosphatonin are to be expected. Whether a phosphatonin could be a factor involved in the regulation of the rapid release of calcium from bone remains to be investigated.

**SECONDARY HYPERPARATHYROIDISM IN UREMIA**

Secondary hyperparathyroidism (sec. HPT) develops early in renal insufficiency and may affect the function of several organs (24, 29, 30). The stimuli for the development of sec. HPT of relevance to renal failure are hypocalcemia, hyperphosphatemia and low levels of 1,25(OH)$_2$D (Calcitriol) is an important regulator of the PTH gene and may play a specific role in the control of the glandular mass (160-162). Pharmacological doses of calcitriol decrease PTH gene expression at the transcriptional level by binding of the 1,25(OH)$_2$D$_3$ receptor (VDR) to a vitamin D-responsive element on the PTH gene promoter (163, 164). The levels of calcitriol in mild and moderate uremia are maintained within the normal range at the expense of elevated PTH levels. In uremia low levels of VDR have been demonstrated in the parathyroid cells (5, 13, 165), together with an impaired affinity of vitamin D to its receptor, resulting in a reduced activity of calcitriol (166). Furthermore, uremia per se enhances the stability of PTH mRNA by a posttranscriptional mechanism that decreases the degradation of RNA, independent of changes in circulating levels of calcium and phosphate (18). The factors that are responsible for the degradation of PTH in the parathyroid cell are probably cytosolic endonucleases (159, 167). Such factors are selectively decreased in uremia (18). Other factors that are regulating the secretion of PTH include some of autocrine or paracrine nature (4, 6). Such an autocrine/paracrine regulation might be involved in the induction of sec. HPT (6). The expression of all these factors may be influenced by the chronic uremia (168, 169). As such, hyperparathyroidism associated with chronic uremia results from a combination of functional and structural changes in the parathyroid glands.

**HYPERPLASIA OF THE PARATHYROID GLANDS**

Parathyroid tissue is a discontinuous replicator tissue, which is characterized by a low cell turnover, a low rate of mitosis, and no separate stem cells (8, 170). As estimated by Parfitt the mean life span of normal parathyroid cell is 20 years in humans and 2 years in rats (170, 171). Mitosis can be stimulated by functional demand. The process of cell loss occurs by apoptosis, a programmed cell death. Apoptosis is probably as important as mitosis in regulating the number of functioning parathyroid cells as cell birth and death must be in balance. In human subjects parathyroid growth progresses in response to chronic renal failure through several stages from diffuse hyperplasia to nodular hyperplasia and to formation of adenomas. Diffuse hyperplasia is initiated by hypocalcemia and phosphorus retention and becomes more severe as the result of calcitriol deficiency (21). The next stage is that hyperplasia becomes nodular and the glandular enlargement asymmetrical. The nodules consist of cells that are more closely packed with large nuclei, an increased prevalence of mitosis and depletion of VDR and CaR (17, 56, 172). Detailed histochemical and immunohistochemical studies indicate similarity in gene expression between the cells in each nodule, but differences between nodules (17, 173). Monoclonal growth of the parathyroid cells has been found in a majority of the uremic patients with refractory hyperparathyroidism (174). The genes responsible for monoclonality have not been identified. Apparently, somatic mutations confer a growth advantage to clones of parathyroid cells, which is causing monoclonal growth and nodular parathyroid hyperplasia, although these two phenomena are not strictly linked. The next stage is emergence of an adenoma in one or occasionally more than one of the nodules, as an expression of a mutation in one of the cells that are undergoing the most rapid proliferation. In
some cases there is a loss of a tumor suppressor gene on chromosome 11, a molecular defect with the potential for disrupting the control of the cell cycle (175). The final and least common stage is malignant transformation leading to parathyroid carcinoma, an event reported in 5 patient on long term hemodialysis (176). It seems that at the initial stages development of parathyroid hyperplasia is a regulatory phenomenon, but that it during the progression escapes from normal growth control.

PARATHYROID GROWTH IN EXPERIMENTAL ANIMAL MODELS

Whether the changes, that are seen in human parathyroids, develop as result of long term uremia and whether sec. HPT can be reproduced in experimental animal models are still matters of debate. The rapid increase in parathyroid cell proliferation after subtotal nephrectomy in the rat was first reported half a century ago (177). The increase of parathyroid cell mass in the 5/6 nephrectomy model of the rat is mainly due to enhanced cell proliferation characterized by an increase in cell number (178, 179). Cell hypertrophy appears to play only a minor role, if any at all (176, 178). This is different from the cell hypertrophy and increase in parathyroid gland volume that has been observed in response to induction of hypocalcemia or hyperphosphatemia by diet manipulation in animals with normal kidney function (180). In another model of normal rats on a diet deficient of both calcium and vitamin D parathyroid cell hyperplasia largely prevailed over hyperthyrophy (7). This latter model resembles more closely the uremic situation.

The heterogeneous nature of parathyroid growth is described by Brown et al. (67) in a model of 5/6 nephrectomized rats kept on a high phosphorus diet. He showed that uremic rats on a high phosphorus diet developed nodular hyperplastic glands. In these nodular areas an increase in the expression of markers of cell proliferation was associated with a decrease in the expression of CaR, a picture similar to that, which is described in human parathyroids with nodular hyperplasia (15, 56). In that study, the uremic rats that were on a normal diet developed no hyperplasia of the parathyroids, in contrast to what has been reported by others (67). Thus, although it has been shown repeatedly in the rat that the increase in PTH secretion immediately after 5/6 nephrectomy is intimately associated with the development of parathyroid cell hyperplasia (20, 181, 182), this is not uniformly agreed upon (67). We, therefore, created a model of pure hyperplasia by the implantation of several isogenic parathyroid glands into one rat in order to show that a direct link between hormone secretion and hyperplasia was not obligatory (2).

ROLE OF CALCIUM, PHOSPHORUS AND CALCITRIOL FOR THE PARATHYROID GROWTH IN UREMIA

The precise role of the disturbances in calcium, phosphorus and calcitriol levels for the development of abnormal parathyroid growth in uremia, as well as the precise role of these factors in the regulation of normal parathyroid growth have not been well established. Calcium deficiency together with calcitriol deficiency are probably the most important stimuli for parathyroid hyperplasia, as it has been shown in vivo in uremic rats by Naveh-Many et al. (7). A concomitant decrease in the expressions of CaR and VDR as described in the parathyroid glands of uremic rats (5, 67) and chronic dialysis patients (15, 56, 183) should theoretically enhance parathyroid cell proliferation even further. This was proven indirectly by the observation that administration of a calcium-sensing receptor agonist, NPS-568, or calcitriol led to the suppression of parathyroid cell proliferation in uremic rats (179, 184).

The role of phosphorus accumulation on parathyroid cell growth is documented in several studies in uremic rats (7, 20, 67). Conversely, an early dietary phosphate restriction prevented parathyroid cell proliferation and oversecretion of PTH in uremic rats (14, 22, 181). Furthermore, it has been found in uremic rats that hyperphosphatemia induced parathyroid cell hyperplasia, even when changes in plasma calcium and calcitriol were carefully avoided, pointing towards a direct effect of phosphorus on cell proliferation (7).

THE LIMITATION OF IN VITRO MODELS IN STUDIES ON PARATHYROID CELL PROLIFERATION

At present it is not known whether the effects of calcitriol, calcium and phosphorus on the parathyroid cell growth are directly or indirectly mediated. In vitro studies on parathyroid regulation are compromised by a number of problems in the parathyroid cell culture models (8, 95, 105, 185-188). There exists no appropriate parathyroid cell line. A cell line derived from rat parathyroid cells turned out to secrete PTHrP and not PTH (189, 190). The culture systems of parathyroid cell are characterized by a rapid loss of PTH secretion. Despite the limitation of culture systems with respect to the maintenance of the parathyroid cell phenotype several research groups have attempted to characterize the possible direct effects of calcitriol and calcium on the parathyroid cell proliferation. In vitro, an inhibitory action of calcitriol on parathyroid cell proliferation has been shown in short-term and long-term culture conditions in bovine parathyroid cells (191-193) and in canine and human glands (194). However, calcitriol has also been shown to inhibit cell proliferation and to enhance cell maturation in vitro in different cell systems (195, 196), making the results difficult to interpret. The stimulatory effect of low calcium on parathyroid cell proliferation could not be confirmed in in vitro experiments (191), indicating that the proliferative effect of low calcium is not mediated directly, or that in vitro system is suitable for conclusions on parathyroid cell proliferation. An inhibitory effect of calcium on proliferation has been noted in rat parathyroid glands (197). An increase of the medium calcium content in other models of dispersed bovine parathyroid cells led to an enhancement of cell proliferation or to no effect at all (186, 191, 192).

No studies have been published so far on the effect in vitro of phosphorus on parathyroid cell proliferation, probably due to problems of precipitation of phosphorus and calcium in the culture medium during long term incubation (95).

PROBLEMS OF IDENTIFICATION OF APOPTOSIS IN THE PARATHYROIDs

Parathyroid hyperplasia reflects, as mentioned before, an imbalance between rates of cell proliferation and apoptosis, with apoptosis probably being as important as mitosis for the regulation of the number of functioning cells. As such, parathyroid hyperplasia reflects a disturbed balance between rates of cell proliferation and apoptosis. However, the possible role of dysregulated apoptosis in the development of parathyroid hyperplasia has not yet been established. The problem is that identification of apoptosis in extremely slow growing tissues, such as the parathyroid, has proven not to be easy due to the lack of sufficiently sensitive techniques (8). Therefore, the stimulus for apoptosis in the parathyroids is not known. The number of apoptotic cells in normal human parathyroids is very low, 1/10000 parathyroid cells, as reported by Zhang et al. (198). The same group has, however, previously by using the same TUNEL technique detected very high apoptotic figures, amounting to 1/100 parathyroid cells (199). This discrepancy underlines the methodological difficulty in assessing apoptosis in the parathyroids (8). In rat models three research groups were unable to find evidence of a programmed parathyroid cell death in normal or hyperplastic parathyroid tissue (7, 171, 182). Experimental studies document that parathyroid hyperplasia can easily be prevented, but is poorly reversible (179, 181), at least is its reversal by apoptosis probably an extremely slow process (173). Due to lack of sufficiently sensitive methods we have not tried to estimate apoptosis in the rat parathyroids, even though understanding of apoptosis is of potential relevance for the control of parathyroid hyperplasia. Effective and selective manipulation of the mechanisms leading to programmed cell death might have therapeutic consequences. The use of the cell's
own mechanisms for elimination might be rational, as the side effects can be minimized and as the process can be operated in a specific direction. The condition is that the function of apoptosis is intact and can be induced. In that context it is an important observation by Zhang et al. (198) that parathyroid cells in hyperplasia are able to undergo apoptosis, even though the number of apoptotic cells are extremely low.

**REVERSIBILITY OF SECONDARY HYPERPARATHYROIDISM**

**POSTTRANSPANT HYPERCALCEMIA AND PERSISTENT HYPERPARATHYROIDISM**

It is of importance in the management of secondary hyperparathyroidism (sec. HPT) to prevent the development of non-suppressible and non-reversible changes in the parathyroid glands. In uremia it is necessary to accept a certain degree of sec. HPT, as enhanced PTH levels early in uremia have important homeostatic functions by stimulating the 1α-hydroxylase activity and by increasing the renal phosphorus excretion (200-205). In more advanced uremia the development of adynamic bone disease should be avoided and, therefore, also in this condition it is necessary to accept some degree of sec. HPT (25, 41, 42, 44). Thus, sec. HPT should be controlled at a level that permits normal bone turnover (25, 41, 42). Ideally, a successful kidney transplantation corrects the abnormalities of mineral metabolism that during uremia lead to sec. HPT and renal osteodystrophy. This includes reversal of uremia, abolition of hypercalcemia, hyperphosphatemia and acidosis, restoration of calcitriol production and reversal of skeletal resistance to PTH and vitamin D (2).

The incidence of hypercalcemia after kidney transplantation is non-negligible and varies between 8.5 and 65%. Among kidney transplanted patients 1.3 to 20% will later have to undergo parathyroidectomy (PTX) (34, 35, 206-209). The natural evolution of hyperparathyroidism after successful kidney transplantation is, in most cases, a spontaneous resolution (40, 206, 210). The pathogenesis of post-transplant hyperparathyroidism is not necessarily due to persistent hyperparathyroidism. Several factors, such as resolution of soft tissue calcifications, immobilization, high doses of corticosteroid treatment and hyperphosphatemia might all contribute (40, 211). Only a minority of the transplanted patients requires PTX (40, 206). The criteria for PTX after kidney transplantation are in most centers symptomatic hypercalcemia or asymptomatic hypercalcemia with inappropriately elevated levels of PTH one or more years after the successful kidney transplantation with normal kidney function (40, 206). The risk of developing post-transplant HPT increases with the duration of dialysis (206, 212-214) and with the severity of the pre-transplant HPT (32, 212, 215). As such, the degree of parathyroid hyperplasia is believed to determine the ability of the parathyroid glands to involute after transplantation (48). One would expect patients requiring PTX to have the most severe changes of the parathyroids, such as mononodal nodular hyperplasia and formation of adenomas. Histological examination of parathyroid glands that were removed due to post-transplant persistent severe HPT revealed, however, diffuse hyperplasia in most cases (216-218) and only rarely adenomas (213, 216-218). This is in contrast to the findings in glands removed from uremic patients, where nodular hyperplasia was the most prevalent (17, 173, 219). The reason for this rather surprising finding is not quite clear. Possibly, molecular studies are required to detect for monoclonality (8, 174).

In the clinical situation, the majority of patients with sec. HPT will, despite previous long-term uremia, present a significant fall in plasma PTH after kidney transplantation. Some part of this fall measured in plasma PTH might be due to clearance of C-terminal PTH fragments as a result of the improvement in GFR, as most of the assays which have been used until recently co-measure some long C-terminal fragments (220-222). In most cases plasma PTH return to near normal by time, although not all studies are confirmative (223). The normalization of GFR seems, however, to be decisive for the normalization of the PTH levels (33, 209, 215, 224). In transplanted patients with reduced GFR elevated PTH levels are to be expected, due to the degree of uremia and independently of whether the patients are transplanted or not. Further experimental studies are needed in order to improve our understanding of the fate of sec. HPT after reversal of uremia by transplantation.

**REVERSAL OF SECONDARY HYPERPARATHYROIDISM AFTER EXPERIMENTAL KIDNEY TRANSPLANTATION**

A model of reversible uremia was created by performing an isogenic kidney transplantation in long-term uremic rats (1, 2). In these uremic rats sec. HPT was induced in two models: a) where the stimulus for the development of moderate sec. HPT was long-term uremia, and b) where severe sec. HPT was induced by long-term uremia, combined with severe hypocalcemia and severe hyperphosphatemia (2). Our results clearly showed, that experimental sec. HPT due to long-term uremia was reversible very rapidly after reversal of the uremia by the experimental isogenic kidney transplantation. Even severe sec. HPT due to long-term uremia associated with hypocalemia and hyperphosphatemia was reversible very fast (2). In both model a and b the circulating levels of PTH became normal as early as within one week after normalization of the kidney function, the plasma-calcium and plasma-phosphorus levels (Figure 3). The precise mechanism behind this rapid reversal of sec. HPT after normalization of GFR is not completely clarified. Some possible pathogenetic mechanisms are listed in Table 1.

![Figure 3](image-url)

**Figure 3.** Kidney function and basal PTH levels before introduction of uremia by 5/6 nephrectomy (2-20 weeks), during uremia (20 weeks to 0 weeks), and after reversal of uremia by an isogenic kidney transplantation (TX). The lines (——) depict kidney function expressed as plasma urea and the bars (■) depict PTH levels in (A) a model of moderate sec. HPT in uremic rats on a standard diet, and in (B) a model of severe sec. HPT in uremic rats on a high P diet. N=12. Mean ± SEM. Modified from reference (2).
Table 1. Regression of sec. Hyperparathyroidism after kidney transplantation. Possible intraglandular mechanisms.

- Inhibition of PTH gene
- Degradation of PTHmRNA
- Increased sensibility to extracellular Ca
- Regulation of Ca expression
- Regulation of VDR expression
- Regulation of VDR/DNA interaction
- Regulation of intracellular PTH degradation
- Regulation of excytosis
- Regulation of the length of the secretory/non-secretory cycle of the single parathyroid cell

PARATHYROID MASS

AN UNSOLVED QUESTION ON THE REGRESSION OF PARATHYROID HYPERPLASIA

An important question is, whether normalization of parathyroid function is associated with regression of parathyroid hyperplasia and whether a direct link between PTH hypersecretion and hyperplasia is obligatory. Can the increased glandular mass – during uremia – again rapidly be reduced? Such a reduction would call for massive apoptosis to take place in the parathyroids. This question has not yet been solved. Examination for apoptosis in the parathyroids is not an easy task (8). Apoptosis would be an attractive explanation for the normalization of PTH secretion after reversal of experimental uremia, at least if the normalization was related to some reduction of the glandular mass. The parathyroids have, however, an extremely low cell turn-over and therefore probably a poorly developed program for cell deletion. There are no known stimuli for apoptosis in the parathyroid cell (8). Vitamin D can still not completely be excluded as a stimulus for apoptosis in the parathyroids (225-227). Evidence for induction of massive apoptosis by calcitriol is indirect and uncertain. In a study by Fukagawa et al. (226) in patients on long-term dialysis oral calcitriol pulse therapy induced a 40% reduction of the parathyroid volume, as measured by ultrasound after 12 weeks of treatment, with most of the volume reduction occurring during the first 4 weeks. The results of the ultrasound method, which was used, was reproducible, but its accuracy is unknown. In 1977 Henry et al. (225) studied parathyroid glands of 3 months old vitamin D deficient chicken that received vitamin D replacement. In that study evidence for a reduction of the number of parathyroid cells was clearly shown. This was based upon reduction in glandular weight and similar reductions in protein and DNA content (225). The changes were detectable already within 4 days and could have resulted only from apoptosis. Not all studies have, however, found similar results. In a study on rats by Szabo et al. (179) 10 days of treatment with calcitriol from the 21. day of uremia did not reverse existing hyperplasia. In a clinical study of Quarles et al. (228) long-term intensive calcitriol therapy failed to decrease the volume of parathyroid glandular mass, as assessed by high resolution ultrasound and/or magnetic resonance imaging. As such, these results are contradictory to the positive results of Fukagawa et al. (226).

Furthermore, when the process of apoptosis was assessed directly by the TUNEL method or by the detection of DNA fragmentation by Jara et al. (229), no apoptosis of the parathyroids was induced in uremic rats, even by extreme high doses of calcitriol. In vitro calcitriol has been shown to inhibit both parathyroid cells apoptosis and proliferation (194). The presence of an eventual “overshoot” of vitamin D production immediately after the experimental kidney transplantation, which might act as a stimulus for apoptosis was not studied in our investigation (2). It seems however very unlikely, that such a phenomenon would result in “pharmacological” levels of vitamin D. Furthermore, it seems unlikely that a process of massive apoptosis was induced by the reversal of uremia, as the size of the parathyroid glands remained large after the experimental kidney transplantation (2). Due to lack of sufficiently sensitive methods we did, as mentioned before, not try to estimate apoptosis in these rat parathyroids. Instead, we used another approach and examined whether an increased parathyroid mass is controllable, and as such, whether non-uremic animals can maintain normal circulating PTH levels despite a considerable hyperplasia of the parathyroids (2).

A MODEL OF PURE PARATHYROID HYPERPLASIA

A model of pure parathyroid hyperplasia was introduced by isogenic implantation of 20 normal parathyroid glands into the neck muscles of normal recipient rats (2). A transient period of hypercalcemia occurred initially after the increase of the parathyroid mass. Within 2 weeks, however, plasma calcium returned to normal levels and remained normal for the following 6 weeks of observation (2). PTH levels were normal from the third day after implantation of the 20 parathyroid glands and remained normal for the following 6 weeks (Figure 4) (2).

Subsequently, parathyroid function was examined and normal suppressibility by calcium of PTH secretion was demonstrated in the rats with 20 parathyroid glands implanted (2). It is not known, whether the transient period of hypercalcemia that was observed, when the parathyroid mass was increased by implantation of several glands into a single rat, is a regulatory phenomenon in the process of downregulation of PTH secretion. As yet we have not characterized the mechanisms behind this transient period of hypercalcemia. One might speculate, whether implantation of several glands into one rat creates a transient degree of relative HPT, as the “normal PTH levels” were higher than expected at the corresponding plasma calcium levels. It’s further a question, whether the posttransplant hypercalcemia, that often is occurring in the human clinic, has a role in the downregulation of the function of the hyperplastic glands. In our model of experimental kidney transplantation no such hypercalcemia was, however, observed (2). The important observations from the model of parathyroid hyperplasia are that both calcium and PTH levels eventually became normal and that this normalization took place when the increased parathyroid mass consisted of normal as well as of uremic parathyroid glands (2). Therefore, these results clearly demonstrated that parathyroid hyperplasia can be controlled in a non-uremic animal.

THE SYNDROME OF “HUNGRY BONE” IS NOT OBSERVED AFTER EXPERIMENTAL KIDNEY TRANSPLANTATION

Another important observation from our study was that no “hungry bone” phenomenon was developed in the experimental kidney transplantation model. The “hungry bone” syndrome is commonly
occurring early after total or partial parathyroidectomy in patients where severe primary or secondary hyperparathyroidism markedly
have accelerated skeletal remodeling (230, 231). The pathogenetic
mechanisms for this phenomenon are not completely clear. Presum-
ably, removal of the excess PTH rapidly reduces the resorptive activ-
ity of the skeleton, while bone formation continues at an increased
number of remodeling sites. Intensive skeletal uptake of calcium fol-
ows, resulting in hypocalemia and in a compensatory increase of
PTH secretion by the remaining parathyroid cells – if subtotal PTX
has been performed. The "hungry bone" syndrome occurs in ure-
mic patients with sec. HPT who are undergoing parathyroidectomy,
and experimentally the syndrome was clearly shown in the our stud-
ies on long-term uremic rats that had the parathyroid glands re-
moved (2). In these severely hyperparathyroid rats the hyperplastic
glands were removed and then normal glands were implanted. An
immediate drop in PTH levels resulted in profound hypocalemia
(2). A similar rapid drop in PTH levels occurred after reversal of
uremia by an experimental kidney transplantation. Daily monitor-
ing of plasma Ca\(^{2+}\) levels revealed, however, no hypocalemia, on
the contrary plasma Ca\(^{2+}\) levels increased to normal (2). After kidney
transplantation the skeletal resistance to PTH, which is associated
with uremia, is lifted (unpublished data), permitting release of cal-
cium from the skeleton. The "hungry bone" syndrome is, however,
common even after partial PTX in patients with primary HPT and
normal kidney function (231). Thus, reversal of uremia might have
additional effects on the skeleton that are not only related to the re-
versal of sec. HPT. Absence of the "hungry bone" syndrome and hy-
pocalcemia after kidney transplantation might be of importance for
the control of the hyperplastic parathyroid glands after kidney
transplantation.

THE CALCIUM/PTH RELATIONSHIP
AFTER EXPERIMENTAL KIDNEY TRANSPLANTATION
The explanation for the rapid normalization of PTH levels after kid-
ney transplantation in the hyperplastic rats from model B (2)
could simply be due to suppression of PTH secretion secondary to
the normalization of plasma Ca\(^{2+}\). This would be in accordance with
the hypothesis that in uremia a PTH secretion is stimulated already at
the actual basal plasma calcium level (45). In the kidney trans-
planted rats we were, however, not able to restore the very high PTH
levels, which were seen in uremia, by an acute and considerable re-
duction of plasma Ca\(^{2+}\) (Figure 2) (2). Thus, we have to conclude that
normalization of circulating PTH levels after normalization of
GFR is not just a simple function of normalization of plasma Ca\(^{2+}\).
Furthermore, HPT rats from model A (2) were not hypocalcemic
before the kidney transplantation, and despite no changes taking
place in plasma Ca\(^{2+}\) a rapid normalization of PTH levels was ob-
served after reversal of uremia. Normalization of plasma phospho-
rus levels may probably play an important role in the normalization of
PTH levels after kidney transplantation. The role of phosphorus in
the regulation of PTH is not yet completely clarified, although evi-
dence has been provided in favor of a direct calcium independent
role of phosphorus in the parathyroid cells (22, 95, 102, 103). The
existence of a sensor/receptor for phosphorus in the parathyroid
cells has, however, not been shown and the molecular mechanisms
involved in the direct effect of phosphorus not yet quite character-
ized. Even severe hypercalcemia in uremic hyperphosphatemic rats
was not able to suppress PTH secretion to the same extent as seen in
normal rats (2). Three weeks after experimental kidney transplanta-
tion normal suppressibility of PTH secretion by calcium was, how-
ever, restored (2). This might be due to return to normophos-
phatemia.

THE PARATHYROID CALCIUM-SENSING RECEPTOR (CaR)
AFTER REVERSAL OF UREMIA
In chronic uremia the abnormal function of the parathyroid glands,
that is resulting in increased PTH biosynthesis and secretion and in
parathyroid cell hyperplasia, is at the molecular level currently re-
lated to disturbances of CaR and VDR and of the regulation of PTH
mRNA (11, 15, 16, 56, 232). Parathyroid glands from patients with
severe secondary or tertiary HPT have elevated set-points for Ca\(^{2+}\)
in vitro (85). A substantial reduction in the expression of the CaR
protein and mRNA has been demonstrated in hyperplastic parathy-
roid glands from uremic patients (15, 56). A decrease of the CaR
protein and mRNA expression has further been observed in the
parathyroid glands of hyperphosphatemic uremic rats, but not in
uremic rats kept on standard diet (5, 19). 1,25(OH\(_2\))D\(_3\) dramatically
decreases PTH gene transcription (162) and might influence the
sensitivity of the parathyroid cells to Ca\(^{2+}\) (79). Changes in the VDR
concentration of the parathyroids would allow for a modulation of
the effect of 1,25(OH\(_2\))D\(_3\). A reduction of VDR protein and mRNA
has been found in glands from uremic patients with severe sec. HPT
(16, 232).

The dramatic decrease of PTH secretion, which took place after
reversal of uremia by an experimental isogenic kidney transplanta-
tion, occurred with unchanged and diminished expression of the
CaR and VDR genes in the parathyroid glands (5). In uremic rats
given a high phosphorus diet the circulating PTH levels were in-
creased 20 times and the CaR mRNA levels decreased by approxi-
ately 60%. Four to 8 days after kidney transplantation the PTH
levels were normalized, while CaR mRNA remained low (Figure 5),
at the level of that in uremic rats (5). Surprisingly, normalization of
the circulating PTH levels after transplantation was not associated with
normalization of the parathyroid CaR gene expression.

In uremia a linkage between CaR and parathyroid cell prolifera-
tion has been suggested. This was based upon the finding that the
calcimimetic compound, NPS R-568, which acts directly on the
CaR, in rats with renal insufficiency inhibited the parathyroid cell
proliferation (182, 184). It remains however unclear, what the mo-
lecular mechanisms are that are involved in the CaR regulated par-
athyroid cell proliferation in uremia. It is furthermore not quite clear,
whether downregulated expression of CaR is inducing proliferation
or whether increased proliferation is inducing downregulation of
CaR. Recent studies might indicate that proliferation of the parathy-
roid gland may precede the downregulation of the CaR (233, 234). A
study examining whether the persistently low CaR expression after
reversal of uremia is associated with changes in parathyroid cell pro-
liferation has to be conducted (5).

The central observation of the study on kidney transplanted rats
was that the CaR mRNA of the parathyroid glands was severely re-
duced despite a dramatic decrease of PTH levels (5). This might indi-
cate the existence of a secretory mechanism in the parathyroid

![Figure 5](image-url)
cells that is not coupled to CaR and which responds to reversal of uremia or to the simultaneous normalization of plasma Ca$^2+$ and phosphorus levels. Support for the existence of such a strong secretory regulation, which is independent of the level of expression of the parathyroid CaR, can be deduced from results by Takahashi et al. (235). They showed in uremic rats on a high phosphorus diet that PTH levels decreased, while the PTH content in the parathyroid cell secretory granules remained high, when phosphorus in the diet – in the same uremic rats – was switched to a low phosphorus content. More recently Ritter et al. in the same model of uremic rats showed that the parathyroid CaR expression was low and remained low immediately after reducing the phosphorus content in the diet (236). The time course of an eventual upregulation of the CaR gene expression in kidney transplanted rats has not been examined. In Ritter et al’s uremic model, however, the CaR mRNA returned to normal after the rats had been on a low phosphorus diet for more than one week (236). The same might be the case in the model of kidney transplanted rats. The question therefore is, whether the regulation of the CaR gene in the parathyroid glands is a slowly conducted process. The CaR is probably an important factor in the “Calciosstat” that determines the plasma Ca$^2+$ level, which the complex calcium homeostasis strives to maintain. Activating and inactivating mutations of the CaR gene are associated with conditions of hypocalcemia and hypercalcemia, respectively (57). Dramatic changes are not to be expected in the “Calciosstat” on a minute-to-minute or day-to-day basis. At present, the regulation of the CaR gene is only sparsely understood.

One might speculate, whether a persistent downregulation of the parathyroid VDR mRNA might contribute to the persistent downregulation of the CaR mRNA, observed after experimental kidney transplantation (Figures 5 & 6) (5). Regulation of the CaR gene by vitamin D has been suggested by Brown et al. (237). They found that the expression of the CaR gene was affected by the vitamin D nutritional status and as such was decreased in rats that were kept on a vitamin D deficient diet. It was shown in the same study that parathyroid CaR mRNA was upregulated by pharmacological doses of 1,25(OH)$^2$D. Recently, Canaff et al. (238) showed that a bolus of 1,25(OH)$^2$D upregulated CaR mRNA in the rat parathyroids already after 12 or 16 hours. Furthermore, a functional vitamin D response element has been identified in the CaR gene (238). This provides understanding of the mechanism by which 1,25(OH)$^2$D is involved in the regulation of the expression of the CaR. In contrast, however, in another study by Rogers et al. (239) no effect of pharmacological doses of 1,25(OH)$^2$D was found on the expression of CaR mRNA levels. Furthermore, in uremic rats that were given a standard diet the VDR gene expression was decreased, while the CaR gene expression was normal (5). Therefore, a link between the activity of 1,25(OH)$^2$D and the expression of the CaR may not be of great significance, or if it exists, such a link may require pharmacological levels of the sterol.

**THE PARATHYROID VITAMIN D RECEPTOR (VDR) AFTER REVERSAL OF UREMIA**

In uremic rat models a reduced VDR mRNA in the parathyroids has been shown (Figure 6) (5,165). A low expression of VDR protein and mRNA has further been demonstrated in hyperplastic parathyroid glands from uremic patients (16,232). It is well documented that 1,25(OH)$^2$D in pharmacological doses is an important inhibitor of the PTH gene (162). It is uncertain whether this is an expression of a physiological role of 1,25(OH)$^2$D in the regulation of parathyroid function. It is also a question, whether the physiological effect of 1,25(OH)$^2$D is directly on the PTH gene or mediated mainly via the calcemic effect of this steroid. Thus, in VDR deficient mice normal circulating levels of PTH and normal histology of the parathyroids could be maintained, as long as the mice were kept normocalcemic by providing them a special rescue diet (240). The control group of untreated VDR deficient mice developed HPT and hyperplasia of the parathyroid glands (240). It has been shown by several investigators (2,9,11) that circulating 1,25(OH)$^2$D levels are normal in uremic rat models. The activity of the sterol might, however, be decreased due to reduced VDR expression in the parathyroid glands.

After experimental kidney transplantation of previously uremic rats it was surprising that the circulating levels of PTH became normal, despite low and unchanged expression of the VDR mRNA (5). Brown et al (241) previously showed that calcium regulates the expression of VDR mRNA in rat parathyroids. Thus, they found that vitamin D depleted rats, which received a high calcium containing diet, had VDR mRNA levels similar to that of rats maintained on a normal vitamin D containing diet. The authors suggested that the upregulation of VDR mRNA in the parathyroid glands by pharmacological doses of 1,25(OH)$^2$D was mainly due to increased plasma calcium levels (241). This concept has recently been supported by the results of Garfia et al. (242), who in vitro showed that VDR expression in the parathyroids was regulated by extracellular calcium. In the study on rats after kidney transplantation plasma Ca$^2+$ levels were normalized, while the VDR mRNA levels in the parathyroids were persistently decreased (5). This difference could be due to the fact that normalization of plasma Ca$^2+$ after experimental kidney transplantation took place very rapidly (2), in contrast to the response of chronic dietary treatment or to the pharmacological effect of 1,25(OH)$^2$D. Another possible explanation is that despite persistently low VDR expression, the activity of 1,25(OH)$^2$D in the parathyroid glands of kidney transplanted rats was improved due to cessation of hypocalcemia (242,243). Thus, it has been reported that nuclear calreticulin in the parathyroids prevents the binding of the VDR-Retinoid X-receptor to the PTH gene during hypocalcemia, and thereby inhibits the downregulatory function of the sterol (243). Thus, recent results underline the complexity of the coordinated regulation of the function of the parathyroid glands by calcium and 1,25(OH)$^2$D.

**PARACRINE - AUTOCRINE FACTORS IN THE REGULATION OF PTH SECRETION**

PTH, Chromogranin A (CgA), Chromogranin A related peptides and Endothelin-1 have all been suggested as factors that might influence secretion of PTH (244-246). The parathyroids are not controlled by a superior “hypothalamic-pituitary axis” as seen in other endocrine glands. Therefore, the parathyroid glands are likely to use autocrine/paracrine regulatory mechanisms. However, only a few studies exist on the autocrine/paracrine regulation of parathyroid function. The status of these factors is described below. We have ex-

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**Figure 6.** VDR gene expression in the parathyroid glands of normal control rats, uremic rats on a high P diet (CRF hP) and kidney transplanted (TX), previously uremic rats in which uremia was reversed by an isogenic kidney transplantation. N=8-7. Mean ± SEM. * P<0.001 versus control rats. Modified from reference (5).
CHROMOGGRANIN A

CgA is an acidic glycoprotein of about 450 amino acids, which together with PTH is a major secretory product of the parathyroid glands (247). CgA is co-stored and co-secreted with PTH (247, 248). Fascito et al. (244) showed that CgA strongly inhibited low calcium-stimulated PTH secretion in cultured porcine parathyroid cells, while addition of antisera to CgA potentiated the stimulatory effect of low calcium on PTH secretion. PTH secretion was, however, not inhibited during a short term incubation time of 1 hour, but only after a longer incubation of 3 hours. This suggests that the protein during the incubation was processed to an active form (249). An early stage in the processing of CgA is many endocrine tissues the generation of a fragment with an apparent molecular weight of 64 kDa (CgA 64). The proteolytic enzyme(s) responsible for cleavage of CgA is not known. In a recent study, Fascito et al. (250) showed that the proteolytic processing of CgA to CgA 64 did not occur in intact secretory granules of porcine parathyroid cells.

In the parathyroid cells CgA secretion and gene expression are regulated by 1,25(OH)2D (251, 252). Thus, in bovine parathyroid cells, 1,25(OH)2D up-regulates CgA mRNA levels in vitro and at the same time down-regulates PTH mRNA (251, 252). Exposure to high or low calcium had no effect on CgA mRNA (252). Soliman et al. (169) confirmed in an in vivo study on rats the reciprocal regulation of CgA and PTH mRNA's by 1,25(OH)2D. This study (169) also examined the CgA mRNA expression in uremic rats – after 5 weeks of CRF, resulting in sec. HPT, normocalcemia and reduced 1,25(OH)2D levels. The parathyroid CgA mRNA levels were 50% lower in uremic rats than in sham operated rats. This suggests a physiological relevance of the earlier in vitro observations.

CgA still requires time consuming proteolytic processing and is, therefore, probably not an autocrine factor involved in the regulation of the secretion of PTH. Thus, more information is needed on the CgA processing in the parathyrroids and on the significance of the different regulations of CgA and PTH.

CHROMOGGRANIN A RELATED PEPTIDES

CgA has been proposed to play a role as a prohormone for smaller biologically active peptides that may function as autocrine or paracrine regulators of PTH secretion (245, 250). One of these CgA-related peptides is pancreastatin that is a 5-kDa amidated peptide (253). The amino acid sequence of pancreastatin is contained within that of CgA. It has been shown by Fascito et al. (254) that exogenous pancreastatin in concentrations as low as 0.1 nM inhibits low calcium-stimulated secretion of both PTH and CgA from dispersed porcine parathyroid cells. Such an inhibitory effect of exogenous pancreastatin was later confirmed by Dress and Hamilton, who, however, were unable to detect pancreastatin in extracts of the secretory granules or in the incubation medium and therefore, questioned the physiological importance of pancreastatin in the bovine parathyroid glands (255).

A stable N-terminal 26-kDa fragment of CgA, which resulted from endogenous breakdown of intact bovine parathyroid CgA and which was found to be secreted by bovine parathyroid cells and generated by parathyroid secretory granules, was further described by Dress et al. (256). This 26-kDa protein inhibited in short term incubations of 60 minutes the low calcium-stimulated PTH secretion and the secretion of CgA to the same extend, as that induced by a high calcium concentration (256).

Parastatin is a peptide, generated by proteolysis of porcine CgA (245, 257). It inhibits low Ca2+-stimulated PTH secretion from dispersed parathyroid cells (257) and immunoreactive parastatin has been detected in extracts of the glands and in the incubation medium (245, 257).

The physiological importance of these CgA-related peptides on PTH secretion and especially on the acute regulation of PTH secretion remains to be clarified.

ENDOTHELIN-1

Endothelin-1 (ET-1) is a potent vasoconstrictive peptide, which participates in the regulation of growth and function of various endocrine tissues. Fujii et al. (246) showed that ET-1 mRNA is synthesized in rat parathyroid epithelial cells and in bovine parathyroid chief cells. Human parathyroid tissue, both adenoma and hyperplasia, expresses preproET-1, and ETA and ETB receptor subtypes (258). ET-1 induces an inhibition of PTH secretion in 2 hour incubations of dispersed bovine parathyroid cells (259). The effect of ET-1 on parathyroid cells is “coupled” to intracellular calcium signaling, as ET-1 caused a dose-dependent rapid elevation of intracellular Ca2+ (129, 259). In hypocalcemic rats, cell proliferation and ET-1 immunoreactivity increase in parallel in the parathyroid cells, an effect which can be blocked by an ET-1 receptor antagonist (260). The possible physiological effect of ET-1 on PTH synthesis and secretion has, however, not yet been evaluated.

PTH/PTHrP RECEPTOR TARGETING MOLECULES AS PARACRINE/AUTOCRINE FACTORS

EVIDENCE FOR PTH RECEPTORS IN THE PARATHYROIDS

The PTH/PTH-related peptide (PTHrP) receptor has been demonstrated by immunohistochemistry in human parathyroid tissue by Matsushita et al., who showed that this receptor was distributed along the cytoplasmic membrane of the parathyroid cells (173). Recently, our group have demonstrated PTH/PTHrP receptor mRNA in rat parathyroids (6). This supports the possibility, that N-terminal PTH and PTHrP, as ligands for the receptor, might regulate parathyroid cell function. Furthermore, it has previously been proposed by Inomata et al. that C-terminal PTH fragments might have an autoregulatory role in the parathyroids (261). In their study on the characterization of a novel PTH receptor with specificity for the C-terminal region of PTH 1-84, several cell lines were screened and significant binding of C-terminal PTH was observed in two cell lines, one of them was a rat parathyroid cell line (PT-r3) and the other one an osteoblast like cell line (261). Metabolism of PTH, both within the parathyroid gland and peripherally, involves cleavage within the region 33-43 of PTH, which potentially could generate biologically active fragments (262). The intracellular degradation of PTH within in the parathyroid cells appears to be regulated (69, 70, 92). When secretion is stimulated by low ambient calcium most of the hormone is in the form of the native molecule. By contrast, when the secretion is suppressed by high ambient calcium most of the secreted hormone consists of fragments (69). Thus, PTH might act as a polypeptide hormone at the level of bone (263) and at the level of the parathyroid cells, as different PTH fragments, secreted from the parathyroids, might have autoregulatory roles. It is, however, not known, whether functional parathyroid tissue expresses the putative C-terminal PTH receptor, at all. No one has yet been able to demonstrate the PTH type-2 receptor in parathyroid cells (264). The expression of the PTH/PTHrP receptor has to be confirmed by more studies. Likewise, it is necessary to examine the functional properties and the regulation of the PTH/PTHrP receptor in the parathyroids.

THE QUESTION OF CIRCULATING PTH AS A REGULATOR OF PTH SECRETION FROM THE PARATHYROIDS

A direct inhibitory effect of PTH 1-34 on PTH secretion from bovine parathyroid primary cultured cells, incubated for only 20 minutes, was first reported by Fujimori et al. (265). PTH 1-34 in concentrations as low as 10-12 M was found efficiently to inhibit the low calcium-stimulated PTH release. At normal Ca2+ concentrations PTH 1-34 suppressed the PTH secretion to levels obtained at high Ca2+ concentration, but no further. The authors suggested, that amino-terminal PTH fragment(s) in the circulation might act in
vivo as physiological inhibitor(s) of the PTH secretion and, thereby, exert a negative feedback mechanism on the regulation of PTH secretion (265). A major difficulty in examining the role of different PTH fragments on PTH secretion, and as such in the interpretation of the results of Fugimi et al., is the cross-reactivity of PTH fragments in the PTH assays. We have tested the hypothesis of Fugimi et al. (265) in a simple in vivo model and found no sign of a negative feedback regulation of circulating PTH on PTH secretion (2). An acute unilateral parathyroidectomy was performed in rats just before initiation of an EGTA infusion or b. during the EGTA infusion. Thus parathyroid mass was reduced by 50%. In these two models circulating PTH levels were reduced by 50%, corresponding to the reduction in parathyroid mass, and remained stable at this level, indicating that the single parathyroid gland that was left in situ did not have an immediate response to changes in circulating PTH, as no compensatory up-regulation of PTH secretion was observed. Thus, the results from our group did not support the existence of a negative feedback action of circulating PTH (2).

N-TERMINAL PTHrP, USED AS A SURROGATE FOR PTH, ENHANCES THE SECRETORY RESPONSE OF PTH TO HYPOCALCEMIA

It could, however, not be excluded from our investigation (2) that PTH might have a paracrine/autocrine role in higher concentrations at the glandular level. Therefore, we decided to use N-terminal fragments of PTHrP instead of PTH in order to activate the common PTH/PTHrP receptor, as the N-terminal PTHrP fragments are equipotent to N-terminal PTH as ligands for the receptor (4). The immunoreactivity of the two hormones is, however, different and PTHrP is not detected by the PTH immunoassays (4). As such the amount of PTHrP added will not be measured as a PTH response. Our results were quite surprising, as no inhibitory effect of PTHrP was seen, but on the contrary, a highly and significant stimulatory effect was demonstrated on PTH secretion (4). Both exogenous PTHrP 1-40 and PTHrP 1-86 dramatically, by approximately 300%, enhanced the secretory response of PTH to a hypocalcemic stimulus in vivo in rats, suggesting the possible existence of an autocrine/paracrine stimulatory function of peptides targeting the PTH/PTHrP receptor in the parathyroid glands (Figure 7) (4). This effect was clearly dose related with increasing doses of PTHrP resulting in an enhanced PTH response to hypocalcemia (6). We ensured that this response was not due to altered peripheral metabolism of PTH, which theoretically could be affected by PTHrP. Synthetic human PTH was injected together with PTHrP or vehicle into the rat and plasma levels of human PTH were measured by the intact human PTH IRMA assay that is not crossreacting with rat PTH. The disappearance of human PTH 1-84 was not affected by PTHrP (4).

The stimulatory effect of PTHrP on PTH secretion took place only in the condition of hypocalcemia. This was shown in vivo experiments, where hypercalcemia first was induced, then a bolus of PTHrP or vehicle was given and plasma Ca++ was then gradually reduced from the level of hypercalcemia to hypocalcemia by a continuous EGTA infusion. During hypercalcemia and in the normocalcemic range no significant difference was found between the PTH levels of the two experimental groups. During hypocalcemia, however, plasma PTH levels became significantly enhanced by PTHrP 1-40 (4).

A direct effect of PTHrP on the parathyroids was further confirmed by in vitro experiment on whole rat parathyroid glands. PTH secretion was significantly stimulated by a low calcium concentration of 0.6 mM and PTHrP 1-40 significantly enhanced the low-calcium stimulated PTH secretion even further (Figure 8) (4).

THE POSSIBILITY FOR A SPECIFIC ROLE OF PTHrP AS A PARACRINE/AUTOCRINE FACTOR IN THE PARATHYRIDS

PTHrP was discovered in 1987 as a humoral factor responsible for humoral hypercalcemia of malignancy (266). Many research groups have now begun to focus on the normal physiological functions of PTHrP (113, 267-271). The PTHrP gene is expressed in essentially every tissue and organ at some point in the fetal development or in adult life (268, 272, 273). PTHrP is expressed in the parathyroids from fetal to adult life in different species (274-276). PTHrP mRNA...
is expressed in normal human, bovine and rat parathyroid tissue (173, 273, 277, 278). As such, the expression of PTHrP in the parathyroids is not only a feature of neoplastic transformation, although an abnormal expression of PTHrP mRNA and protein has been demonstrated in human parathyroid adenomas, hyperplasia and carcinomas (273, 279, 280). PTH and PTHrP are found to be co-localized in the same secretory granules and secreted simultaneously from parathyroid adenomas (281). In human parathyroid hyperplasia secondary to chronic renal failure (173) a majority of the glands were found to be positive for PTHrP staining by immunohistochemistry both in the diffuse and nodular type of hyperplasia. A similar distribution of PTH-PTHrP mRNA was found (173). A double immunofluorescent staining for PTH and PTHrP revealed distinct differences in parathyroid nodular hyperplasia (173). Thus, it was shown that each nodule homogeneously comprised of one type of parathyroid cells, that were either exclusively PTH positive, exclusively PTHrP positive, or positive for PTH as well as for PTHrP (173). The proliferative activity of the parathyroid cells, as evaluated by the expression of the proliferating cell nuclear antigen, correlated negatively with the expression of PTHrP. Therefore, an inhibitory effect of PTHrP on parathyroid cell proliferation was postulated (173).

PTHRP exhibits biological similarities to transforming growth factor-β (TGF-β) and may have effects on cell growth or differentiation (267). Our results, which clearly showed a stimulatory effect of PTHrP on secretion of PTH, further support the possibility of an autocrine/paracrine function of PTHrP in the parathyroids (4). However, the physiological role of PTHrP in the parathyroids remains to be established.

A POSITIVE AUTO FEEDBACK REGULATION IN THE PARATHYROIDS

Based upon our results, which demonstrated that N-terminal PTHrP very fast, within 5 minutes, and in a dose-related manner enhanced the PTH secretory response to hypocalcemia, we would like to propose a model for the existence of a positive auto feedback regulatory mechanism of N-terminal PTH on its own secretion (6). PTH is secreted by parathyroid glands, there are PTH/PTHrP receptors on the parathyroid cells (6, 173) and PTH is an equipotent ligand to PTHrP for this receptor (282, 283). Thus, we hypothesize that PTH in a hypocalcemic condition enhances its own secretion. We have, however, not yet been able to prove this theory directly in our rat model, as the existing rat PTH assays are co-measuring other mic patients with severe sec. HPT. In these in vitro experiments a significant stimulatory effect of rat PTH 1-34 on PTH secretion, as measured by an intact human PTH assay, was clearly demonstrated (unpublished data). This observation strongly supports the hypothesis of a positive auto feedback regulatory role of PTH. Furthermore, a significant stimulatory effect of PTHrP 1-40 on PTH secretion was found in human hyperplastic parathyroid glands, too. Thus, this is not only a rat phenomenon, as human parathyroid tissue is responding to stimulation of the PTH/PTHrP receptor.

In long-term incubations of 6 hr. with PTHrP an increase of PTH mRNA was seen, and furthermore, it was shown that stimulation of PTH secretion by PTHrP simultaneously had an effect on the arachidonic acid (AA) concentration in the human parathyroid cells (manuscript in preparation).

The possible existence of a positive feedback mechanism will allow for explanation of several phenomena in the parathyroid glands.

The suppression of PTH secretion by Ca²⁺ is mediated via activation of the Ca-sensing receptor on the parathyroid cell surface (57). How low Ca²⁺ stimulates PTH release from the parathyroid cells remains obscure. Our observation, that the activation of the PTH/PTHrP receptor increases PTH secretion by several fold, but only during hypocalcemia opens up for the possibility of amplification of PTH release by PTH itself, when an increased level is needed (6). Cultured parathyroid cells in contact with other's parathyroid cells have been shown to secrete more PTH than isolated parathyroid cells, when exposed to the same stimulus (95). This result has not been explained, but could be mediated by PTH from one cell stimulating further release from the neighboring cells. Finally, a transient nature of the initial increase of PTH levels in response to a reduction in plasma Ca²⁺ has been described in human subjects and in rats (6, 88). A rapid and pronounced decrease in plasma Ca²⁺ is followed by an initial rapid increase in plasma PTH level, which soon declines to a new lower level, despite a continuous fall in plasma Ca²⁺. It is reasonable to speculate, that a positive feedback would cause augmented secretion during the initial stages of elevated PTH levels, giving rise to a greater bolus of PTH release. Then, however, other mechanisms may eventually take over to suppress the PTH release (6, 244, 259, 284).

Autoreceptor effects on hormone or neurotransmitter secretion are well known. Most autoreceptors mediate, however, a negative feedback on the secretion (52, 285-287). We are, however, not alone with the theory of the existence of a positive autoregulatory feedback mechanism relating to PTH, as the pancreatic β-cell-insulin system appears to be a rare example of such a positive feedback of insulin on the secretion of insulin (50). Several lines of evidence support the possibility of an autocrine action of insulin on the β-cell response. Insulin-stimulated insulin secretion has been demonstrated in single pancreatic β-cells (53). A functional insulin receptor and receptor substrates have been identified in both clonal and primary β-cells (288, 289). The response to both insulin and glucose was blunted, when the insulin receptor was not present (290). Recently, it has been shown, that selective insulin signaling through insulin receptors regulates the transcription of the insulin gene in pancreatic β-cells (290). Furthermore, an overexpression of the insulin receptor in the β-cells positively regulates insulin gene expression and leads to increased insulin content in this cell type (50). Thus, a positive auto-feedback regulation in the parathyroid glands might be an expression of a more general endocrine mechanism.

SUMMARY AND CONCLUSIONS

The understanding of the regulation of parathyroid function is of significant importance for the nephrologists, as chronic uremia is associated with severe disturbances in the mineral ion homeostasis that are progressing during the course of uremia and that often are persisting after kidney transplantation. The secondary hyperparathyroidism (HPT) in uremia is characterized by an increase in PTH gene expression, an increase in PTH synthesis and secretion and with proliferation of the parathyroid cells. At the molecular level the abnormal function of parathyroid glands in chronic uremia is currently related to disturbances of the calcium-sensing receptor (CaR), of the vitamin D receptor (VDR), and of the posttranscriptional regulation of PTH mRNA in the parathyroid cells.

In the present investigations the parathyroid function was examined in experimental rat models, where sec. HPT was induced by partial nephrectomy and by different dietary manipulations. An unique model of reversible uremia was created by performing an experimental kidney transplantation in previously long-term uremic rats. In this model chronic uremia was reversed by the isogenic kidney transplantation, permitting examination of the reversibility of the sec. HPT without any interference from immunosuppressive treatment. The parathyroid glands were removed by microsurgery for examination of the expressions of the CaR and VDR genes in uremia and after kidney transplantation.

In addition a model of parathyroid hyperplasia was created by implantation of several normal or uremic isogenic parathyroid glands.
into one rat. The Ca\textsuperscript{2+}/PTH relationship was studied in normal and uremic rats, in kidney transplanted rats and in the model of parathyroid hyperplasia.

Experimental sec. HPT was developed due to long-term uremia and severe sec. HPT was induced by long-term uremia in rats on a high phosphorus diet. Even severe sec. HPT, which was due to long-term uremia and associated with hypocalcemia and hyperphosphatemia, was reversible very fast after reversal of uremia by the experimental isogenic kidney transplantation. In both models of sec. HPT the circulating levels of PTH became normal very early, within one week after normalization of kidney function and of plasma-calcium and phosphorus levels. The precise mechanism behind this rapid reversal of sec. HPT is not completely clarified. A reduction of the CaR and VDR mRNA was demonstrated in parathyroid glands obtained from uremic rats with severe sec. HPT. The dramatic decrease of PTH secretion, which took place after reversal of uremia by an experimental isogenic kidney transplantation, occurred however with unchanged and diminished expression of the CaR and VDR genes in the parathyroid glands. In uremic rats that were given a high phosphorus diet the circulating levels of PTH were increased 20 times and the CaR mRNA levels decreased by approximately 60%. Four to 8 days after kidney transplantation the PTH levels became normal, despite remaining low expression of CaR mRNA, similar to levels in the uremic rats. Surprisingly, normalization of circulating PTH levels after transplantation was not associated with normalization of the parathyroid CaR gene expression. This might indicate the existence of a secretory mechanism in the parathyroid cells that is not coupled to CaR and which responds to reversal of uremia or to the simultaneous normalization of plasma Ca\textsuperscript{2+} and phosphorus levels. In uremic hyperphosphatemic rats high plasma calcium did not suppress PTH secretion to the extent as seen in normal and uremic normophosphatemic rats. Three weeks after an experimental kidney transplantation normal suppressibility by calcium of PTH secretion was, however, restored.

A model of pure parathyroid hyperplasia was introduced by isogenic implantation of several normal or uremic parathyroid glands into normal recipient rats. A transient period of hypercalcemia occurred initially after the increase of the parathyroid mass. Within 2 weeks plasma calcium returned, however, to normal levels and remained normal for the following 6 weeks of observation. PTH levels were normal from the third day after implantation of the 20 parathyroid glands. Subsequently, parathyroid function was examined and normal suppressibility by calcium of PTH secretion was demonstrated in the rats with 20 parathyroid glands implanted. The important observations from this model of parathyroid hyperplasia are that both calcium and PTH levels eventually became normal and that this normalization occurred despite persistent increased parathyroid mass of normal as well as of uremic parathyroid glands. Therefore, these results clearly demonstrated that parathyroid hyperplasia can be controlled in a non-uremic animal.

The "minimal" or "maximal" levels of PTH in response to suppression or stimulation of PTH secretion by changes in extracellular calcium has traditionally been taken as an expression of the parathyroid glandular mass. The present results provided important new aspects to the Ca\textsuperscript{2+}/PTH relationship by the demonstration of "minimal" PTH levels not being an expression of the parathyroid mass.

In traditional model of the calcium ion homeostasis the emphasis is on the key role of PTH, as the extracellular first messenger maintaining the stable Ca\textsuperscript{2+} concentration in the extracellular fluid. However, a rapid and within minute recovery of plasma Ca\textsuperscript{2+} took place immediately after termination of the induction of hypocalcemia, despite no PTH present in the circulation and despite elimination of the renal handling of calcium in the rat. PTH was, however, setting the level of plasma Ca\textsuperscript{2+}, which is maintained by this complex homeostatic system. Thus, the present results suggest that PTH might not be of significant importance for maintaining the extremely stable extracellular calcium balance and point toward the existence of other factors or mechanisms involved in the rapid minute-to-minute regulation of plasma Ca\textsuperscript{2+}. Future studies may unveil whether new Ca\textsuperscript{2+} sensors or new phosphatonin mediate the regulation of fluxes of calcium between bone and ECF.

The parathyroids are not controlled by a superior "hypothalamic-pituitary axis" and the parathyroids are therefore likely to use autocrine/paracrine regulatory mechanisms. Autoreceptor effects of some hormones, e.g. insulin, have previously been documented. In the present investigation it was examined whether PTH might exert a feedback regulatory effect on its own secretion from the parathyroid glands. The existence of positive feedback will allow for the explanation of several phenomena in the parathyroid glands and add further to the understanding of the Ca\textsuperscript{2+}/PTH relationship. The expression of the PTH/PTHrP receptor gene in the rat parathyroid glands was shown by RT-PCR. The receptor was activated by PTHrP as surrogate for PTH. Both exogenous PTHrP 1-40 and PTHrP 1-86 dramatically, by approximately 300%, enhanced the secretory response of PTH to a hypocalcemic stimulus in vivo in rats, suggesting the possible existence of an autocrine/paracrine stimulatory function of peptides, that are targeting the PTH/PTHrP receptor in the parathyroid glands. This effect was clearly dose related with increasing doses of PTHrP resulting in an enhanced PTH response to hypocalcemia. It was ensured that this response was not due to altered peripheral metabolism of PTH. The stimulatory effect of PTHrP on PTH secretion occurred in vivo, but only in the condition of hypocalcemia and not during hypercalcemia or normocalcemia. A direct effect of PTHrP on the parathyroids was further confirmed by in vitro experiments on whole rat parathyroid glands. PTH secretion was significantly stimulated by a low calcium concentration of 0.6 mM and PTHrP 1-40 significantly enhanced the low-calcium stimulated PTH secretion even further. Our results from in vivo and in vitro experiments demonstrated for the first time that PTHrP significantly enhanced the low Ca\textsuperscript{2+} stimulated PTH secretion. Furthermore, they clearly showed that the level of PTH that previously had been considered as an expression of the "maximal secretory capacity" of the parathyroid cells in fact was not the maximum, but that the "maximal" PTH secretion could be increased even further by several fold. The present results have clearly demonstrated that ligands for the PTH/PTHrP receptor have a significant positive stimulating effect on the secretion of PTH. Thus, the hypothesis is, that these ligands act as surrogate for PTH on the common receptor, and that PTH therefore might have a positive autocrine effect on its own secretion from the parathyroid glands.

IN CONCLUSION:

1. Experimental models for studying the parathyroid function in uremia and after kidney transplantation was established in the rat.
2. Secondary hyperparathyroidism, induced by uremia or by uremia and a high phosphorus diet, was reversible very fast after reversal of uremia by an experimental isogenic kidney transplantation.
3. Parathyroid hyperplasia can be controlled in non-uremic animals resulting in normal circulating levels of PTH and in normal suppressibility of the secretion of PTH by calcium.
4. The dramatic decrease of PTH secretion, which took place after reversal of uremia by an experimental isogenic kidney transplantation, occurred despite unchanged and severely reduced expression of the CaR and VDR genes in the parathyroids. The mechanism behind this rapid reversal of sec. HPT remains to be clarified.
5. The "minimal" or "maximal" levels of PTH, that are obtained during suppression or stimulation of PTH secretion by changes in extracellular calcium, are not an expression of glandular mass.
6. PTH is not the key hormone involved in the rapid minute-to-minute regulation of plasma Ca\textsuperscript{2+}.
7. PTH might exert a positive feedback regulatory effect on its own secretion from the parathyroids by an autocrine paracrine mechanism. PTH/PTHrP receptor gene expression was shown in the rat parathyroids and receptor ligands enhanced the secretory response of PTH to a hypocalcemic stimulus in vivo as well as in vitro.

ABBREVIATIONS
AA: Arachidonic acid
Ca2+: Ionized calcium
CalR: Calcium sensing receptor
CRF: Chronic renal failure
C-terminal: Carboxy-terminal
DNA: Deoxyribonucleic acid
ECF: Extracellular fluid
EDTA: Ethylenediaminetetraacetic acid
EGTA: Ethylene-bis(oxyethylene-nitrilo) tetraacetic acid
ET-1: Endothelin-1
FGF-23: Fibroblast growth factor 23
FHH: Familiar hypercalcaemic hypocalcuria
GFR: Glomerular filtration rate
HPT: Hyperparathyroidism
HPTX: Parathyroidectomy
IP3: Inositol-tri-phosphate
IRMA assay: Immunoradiometric assay
kDa: Kilo Dalton
mRNA: Messenger ribonucleic acid
N-terminal: Amino-terminal
PKC: Protein kinase C
PHEX: Phosphate-regulating gene with homology to N-terminal
PTX: Parathyroidectomized
PTH: Parathyroid hormone
PTH 1-34: Amino-terminal part of PTH
PTH 1-84: Intact parathyroid hormone
PTHrP: Parathyroid hormone related peptide
PTX: Parathyroidectomized
RNA: Ribonucleic acid
RT-PCR: Reverse transcriptase-polymerase chain reaction
Sec. HPT: Secondary hyperparathyroidism
TGF-b: Transforming growth factor-b
TPTX: Thyroparathyroidectomized
TUNEL: Terminal deoxynucleotidyl transferase-dUTP nick end labeling
VDR: Vitamin D receptor
1,25(OH)2D: Calcitriol

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