Histol Histopathol (2000) 15: 619-627
DOI: 10.14670/HH-15.619
http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

Invited Review

Pathophysiology of primary hyperparathyroidism

P. Hellman[1], T. Carling[1], L. Rask[2] and G. Åkerström[1]
Departments of [1]Surgery and [2]Medical Chemistry, Uppsala University, Uppsala, Sweden

Summary. Parathyroid gland is the overall regulatory organ within the systemic calcium homeostasis. Through cell surface bound calcium-sensing receptors external calcium inversely regulates release of parathyroid hormone (PTH). This mechanism, which is voltage independent and most sensitive around physiologic calcium concentrations, is regulated through a 120 kDa calcium sensing receptor, CaR. Inherited inactivation of this receptor is the cause for familial hypocalciuric hypercalcemia (FHH). Parallel research identified the 550 kDa glycoprotein megalin, which also is expressed on the parathyroid cell surface, as another potential calcium sensing protein. Although this protein expresses numerous calcium binding sites on its external domain, its main function may be calcium sensitive binding and uptake of steroid hormones, such as 25-OH-vitamin D_3 (bound to vitamin D binding protein) and retinol. In hyperparathyroidism (HPT), excessive PTH is secreted and the calcium sensitivity of the cells reduced, i.e. the set-point, defined as the external calcium concentration at which half-maximal inhibition of PTH release occurs, shifted to the right. Pathological cells have reduced expression of both CaR and megalin, and reduced amount of intracellular lipids, possibly including stored steroid hormones. A number of possible genetic disturbances have been identified, indicating multifactorial reasons for the disease. In postmenopausal women, however, the individual group with highest incidence of disease, a causal relation to reduced effect of vitamin D is possible. An incipient renal insufficiency with age, lack of sunshine in the Northern Hemisphere, and an association to the baT haplotype of the vitamin D receptor supports this theory. This review summarizes data on regulation of PTH release, dysregulation in HPT, as well as proliferation of parathyroid cells.

Key words: Parathyroid, Calcium receptor, Hyperparathyroidism, Tumor, Hypercalcemia

Offprint requests to: Per Hellman, M.D., Ph.D., Associate Professor. Department of Surgery, University Hospital, **S-751 85** Uppsala, Sweden. Fax: +46-18-504414. e-mail: per.hellman@kirurgi.uu.se

Introduction

The parathyroid glands, the overall regulatory organ within the systemic calcium homeostasis, exert an exceptional control of its principal secretory product, parathyroid hormone (PTH). The most important and rapid regulation is exerted by the ambient calcium concentration, and even minor changes in serum calcium concentrations will cause significant changes in the PTH release (Brown, 1982). Even though many substances have been found to influence PTH secretion, calcium is the most potent one, exerting its effect through cell surface-bound calcium-sensing receptors, with the ability to mediate signal to the cell interior (Brown and Hebert, 1996). The relation between external calcium and PTH release is inversely sigmoidal, where the calcium concentration that causes half-maximal inhibition of the PTH release (e.g., the set-point) is situated in the steepest part of the curve corresponding to the physiological concentration ranges for ionized calcium (Brown, 1983).

Hyperparathyroidism (HPT) is caused by one or several enlarged parathyroid glands with excessive release of PTH. PTH activates its peripheral receptor present in several organs including many without involvement in the classical calcium homeostasis (Juppner et al., 1991). In bone and kidney, however, PTH binding to its receptor yields increased levels of plasma calcium, through increased reabsorption of kidney tubule calcium and increased osteoclast activity causing mobilization of calcium from the bone (Habener et al., 1984). Thus, HPT is characterized by increased plasma levels of calcium and may be diagnosed by determination of relatively increased serum PTH levels (Hellman et al., 1994). This review summarizes aspects on the current understanding of normal parathyroid cell physiology, with special focus on the pathophysiology of HP?:

Regulation of PTH release

Calcium sensing

The dose-response relationship between the external

calcium concentration and the PTH release is inversely sigmoidal but a non-suppressible component of the PTH secretion persists even at high external calcium levels (Brown et al., 1979; Wallfelt et al., 1988a,b). The rapid stimulatory effect of a reduced external calcium concentration mainly affects the secretion of recently synthesized PTH, while long-standing hypocalcemia increases the release also of PTH stored in secretory granules (Habener et al., 1984). On the other hand, acute hypercalcemia rapidly inhibits the PTH release, and also increases the intracellular PTH degradation and reduces the PTH gene transcription (Habener et al., 1984; Okazaki et al., 1992). The concept of cell surface-located calcium-sensing proteins, which monitors the external calcium concentration, was proposed after electrophysiological studies of bovine parathyroid cells, and studies of cytoplasmic calcium ($[Ca^{2+}]_i$), found to act as a principal intracellular messenger in parathyroid cells (Lopez-Barneo and Armstrong, 1983; Gylfe et al., 1986; Nemeth and Scarpa, 1986). These studies revealed that a sudden increase in external calcium induces a transient rise in $[Ca^{2+}]_i$, presumably through release of intracellular stores of calcium, followed by influx of calcium through opening of calcium channels (Larsson et al., 1984). The associated depolarization was demonstrated to be caused by calcium itself, and the calcium channels were found to be voltage-independent supported by findings that the L-type calcium channel blocker verapamil did not affect $[Ca^{2+}]_i$ (Lopez-Barneo and Armstrong, 1983; Ridefelt et al., 1996). The rather unusual voltage-independent depolarization of these cells was the basis for postulation of a cell surface-bound calcium-sensing receptor regulating PTH release (Gylfe et al., 1987). Recent studies using image analysis technology have revealed that increased external Ca^{2+} induces verapamil-sensitive $[Ca^{2+}]_i$ oscillations, and that the frequency of these oscillations relates to the external Ca^{2+} concentration (Ridefelt et al., 1995). Thus, both voltage-independent and voltage-dependent calcium channels seem to be expressed, and there may indeed be several mechanisms for regulation of $[Ca^{2+}]_i$ in the parathyroid cell. The sensor mechanism(s) is not selective for calcium, but also senses other cations, like magnesium and the polyvalent cation neomycin, which both induce transient rises in $[Ca^{2+}]_i$ and inhibit PTH release (Ridefelt et al., 1992a,b).

Research using expression cloning in oocytes resulted in the identification and cloning of a G-protein-coupled 120 kDa glycoprotein with a seven transmembrane receptor-like structure (Brown et al., 1993). This protein, denoted CaR (Calcium Receptor), is expressed in various tissues with known or (yet) unknown calcium sensing properties, like the parathyroid and kidney tubular cells, keratinocytes, certain cerebral cells and thyroid C-cells (Garrett et al., 1995; Ruat et al., 1995; Bikle et al., 1996). Transient expression experiments of full-length CaR cDNA have documented its calcium receptor function and its coupling to $[Ca^{2+}]_i$ as an intracellular messenger. The reverse, knock-out experiment is performed in nature itself, since point mutations or inserts of Alu-repeat in the CaR gene cause variable degrees of inactivation in individuals with familial hypocalciuric hypercalcemia, FHH. Thus, the hypercalcemia and hypocalciuria of FHH are related to a reduced calcium-sensing function from inactivation of the CaR, which leads to a right-shifted set-point for the urinary calcium clearance and the PTH release (Pollak et al., 1993, 1994). Inactivation of both alleles leads to neonatal severe hyperparathyroidism (NSHPT), with substantial mortality unless surgery is performed neonatally.

Parallel research led to identification of the human version of a rat protein referred to as the rat Heymann nephritis antigen, gp330 or LRP-2 in the literature (Lundgren et al., 1994). The human version was initially denoted CAS (CAlcium Sensor), while the subsequently cloned rat protein was called megalin, due to its large size (Saito et al., 1994). In this review we apply the name megalin also for the human version. This large, 550 kDa glycoprotein, has been proposed as another candidate for a parathyroid calcium sensing receptor protein. Murine monoclonal antibodies directed against human parathyroid cells recognized the protein, and one of the antibodies was found to functionally inhibit the Ca^{2+} induced rise in $[Ca^{2+}]_i$ and reduction in PTH release (Juhlin et al., 1987a,b). These results were supported by studies in placental cytotrophoblasts, also demonstrating a potential calcium regulation of release of PTH-related protein (PTHrP) from these cells (Hellman et al., 1992). Further characterization revealed that the human sequence and structure closely resembled rat megalin (Juhlin et al., 1988; Lundgren et al., 1994; Hjälm et al., 1996). This protein has previously mainly been identified as an endocytotic receptor for various protein complexes or apolipoprotein B or J, and has been thought to function merely as a scavenger protein (Farquhar et al., 1994). Interestingly, recent research has identified megalin as an uptake mechanism in the renal proximal tubule for intact or aminoterminal fragments of PTH, 25-OH-vitamin D_3 bound to the plasma vitamin D-binding protein (DBP), as well as retinol, which have been filtrated through the glomerulus (Hilpert et al., 1999; Nykjaer et al., 1999). Megalin has thus been proposed as a receptor effectuating tissue selective uptake of steroid hormones, with implication not only for local calcium homeostasis (Nykjaer et al., 1999). These findings may explain physiological mechanisms also in the parathyroid, where megalin may constitute a mechanism for vitamin D (and possibly vitamin A) regulation of parathyroid chief cell function. Indeed, a cell surface mechanism with receptor-like properties recognizing vitamin D has been proposed in several cells, including the parathyroid (Baran et al., 1991; Bouillon et al., 1995). Moreover, although the PTH/PTHrP receptor seems to be absent on the parathyroid cell surface (our own unpublished results), aminoterminal PTH(1-34) inhibits PTH release in cultured parathyroid cells (Fujimi et al., 1991),

implicating a possible role for megalin in a negative feed-back mechanism.

In addition, the initial studies in parathyroid cells and placental cytotrophoblasts mentioned above, as well as the additional selective tissue expression of megalin, (e.g. proximal tubular brush border, placental cytotrophoblasts, type II pneumocytes and mammary epithelium) support its action as a calcium-sensing receptor (Juhlin et al., 1990; Hellman et al., 1992; Lundgren et al., 1997). However, many tissues including parathyroid express CaR and megalin concomitantly, whereby the calcium sensing mechanism may depend on expression of both proteins. However, there are examples of tissues and cells expressing only one of the proteins, but still demonstrating calcium-sensing properties. Thyroid C-cells express CaR but not megalin, but display calcium-sensitive regulation of $[Ca^{2+}]_i$ oscillations and calcitonin release (Garrett et al., 1995). On the other hand, a subclone of the rat proximal tubule cell line IRPTC, expresses megalin but not CaR, and these cells do in fact react with parathyroid-like alterations in $[Ca^{2+}]_i$ when exposed to external cation stimuli (Hellman et al., 1995a,b; Tang et al., 1995). However, although several studies indicate that megalin is involved in the calcium sensing, this is still not proven, since proper transfection experiments documenting its role as a Ca^{2+} sensor have not yet been performed, mainly due to technical problems with the large cDNA (~13 kb). The megalin knockout mice may help to clarify this issue, although mice with homozygously disrupted megalin genes die perinatally due to respiratory failure (Willnow et al., 1996).

All available data taken together may thus indicate that CaR is not the only calcium sensing receptor on the parathyroid cell surface, and that megalin in addition may function as a possible sensing and/or uptake mechanism for steroid hormones and PTH, although their respective roles in health and disease awaits further analysis.

Intracellular signaling

Several intracellular factors are involved in further transport of the external cation message in the parathyroid cells. The most studied of these is $[Ca^{2+}]_i$, which relates to the external calcium concentration in a positive sigmoidal fashion. The mechanism for the increased $[Ca^{2+}]_i$ is attributed to activation of phospholipase C and increase in phosphoinositol turnover with the production of inositol triphosphate (IP3) and IP4 (Epstein et al., 1985; Shoback et al., 1988). In cells transfected with CaR, Ca^{2+} activates this cascade (Kifor et al., 1997). However, Ca^{2+} also increases the levels of diacylglycerol (DAG), another product of the activated phosphoinositol turnover (McKay and Miller, 1996). DAG activates protein kinase C (PKC), but several experiments have substantiated the somewhat surprising finding that Ca^{2+} decreases rather than increases the PKC activity, and that activation of PKC at low Ca^{2+} levels decreases PTH release, while such activation at high Ca^{2+} increases the secretion (Ridefelt et al., 1992a,b; Racke and Nemeth, 1993). Recent research may enlighten this area, since high Ca^{2+} has been found to phosphorylate intracellular PKC-activating sites in CaR, mainly Thr888, which in turn may mediate an inhibition of the intracellular Ca^{2+} mobilization by blunting the stimulation of phospholipase C, and thereby increasing the PTH secretion (Bai et al., 1998). In addition, recent discoveries have clarified that besides the classical PKCs, which are activated by both Ca^{2+} and DAG, there are the novel PKCs activated by DAG but not Ca^{2+}, and the atypical PKCs requiring neither of these stimuli (Shivji et al., 1996).

Possible routes whereby external Ca^{2+} stimulus may act via megalin need to be clarified, but the amino acid sequence in the intracellular tail in this large protein has been demonstrated to contain possible SH2-, SH3-, or PTB-domains, thus offering a prerequisite for further protein-protein interactions (Hjälm et al., 1996).

Vitamin A and D

A vitamin D-responsive element (VDRE) consisting of a single hexanucleotide is present in the PTH gene promoter, which mediates the inhibitory effects of vitamin D on PTH mRNA transcription (Demay et al., 1992). The inhibition is documented in vivo in rats (Silver et al., 1985), as well as in vitro in bovine parathyroid cell cultures (Cantley et al., 1985).

Vitamin D has antiproliferative effects in many cell systems (Carlberg and Polly, 1998), and has in parathyroid cells also been demonstrated to inhibit cell replication, both in vivo (Naveh-Many et al., 1995), and in vitro (Nygren et al., 1988). Further, vitamin D_3 is known to exert differentiating effects on many cell systems, including the parathyroid (Delmez et al., 1989). The recently demonstrated link between megalin and 25-OH-vitamin D_3, retinoids and PTH uptake in proximal tubule cells, may also hypothetically function in parathyroid cells (Hilpert et al., 1999; Nykjaer et al., 1999).

Parathyroid cells have been demonstrated to express a complete set-up of proteins required for intracellular handling of retinol and retinoic acid (Liu et al., 1996). Indeed, retinol is metabolized in the parathyroid to all-trans- and 9-cis-retinoic acid (RA), the active ligands for the nuclear receptors (Liu et al., 1996). Retinoic acids, like active vitamin D, have been shown to inhibit PTH mRNA expression, and also to affect the PTH release (MacDonald et al., 1994; Liu et al., 1996). Further, all-trans-, and 9-cis-RA were demonstrated to inhibit proliferation of parathyroid cells to similar degrees as vitamin D (Hellman et al., 1998). The presence of the cellular retinoid-binding proteins, the nuclear receptors, and the RA metabolism in parathyroid cells support the hypothesis that normal parathyroid cells may function as storage cells for lipids, and mediate vitamin D and A

uptake in parallel to the Ito cells of the liver. Whether megalin in addition may effectuate calcium regulation of retinol (and vitamin D) uptake in parathyroid (and other) cells awaits further analysis.

Hyperparathyroidism

Clinical variants

HPT is characterized by hypercalcemia due to excessive PTH secretion from one or several diseased and generally enlarged parathyroid glands. While secondary HPT is most frequently associated with renal failure, primary HPT may be sporadic or familial. Parathyroid adenoma is the most frequent (85%) histopathological entity in non-familial HPT, and this disease is particularly prevalent in the elderly (female) population. On the contrary, onset of familial HPT is earlier and more often caused by multiglandular disease.

Pathophysiology

In the pathological parathyroid tissue, the regulation of PTH release is functionally disturbed. This derangement, or functional dedifferentiation, is characterized by a relative insensitivity of $[Ca^{2+}]_i$ and PTH secretion to changes in the external Ca^{2+}, which results in right-shifted dose-response relationships (or set-points) (Larsson et al., 1984). The degree of right-shift correlates and to a large extent determines the serum Ca^{2+} value of the individual patient (Wallfelt et al., 1988a,b), and seems to be valid, albeit to a variable extent, for virtually all the histopathological entities of primary and secondary HPT. The lowered sensitivity to external Ca^{2+} may theoretically be caused by reduced expression of Ca^{2+} sensors. This is supported by the reduced expression of both CaR and megalin, demonstrated at the mRNA as well as protein levels (Juhlin et al., 1988; Kifor et al., 1996; Farnebo et al., 1997; Lundgren et al., 1997). A similar type of functional dedifferentiation may be induced in cultures of normal bovine parathyroid cells, which develop a gradual right-shift in the set-point for the PTH release in parallel with reduced expression of CaR and megalin mRNAs (Nygren et al., 1988; Kifor et al., 1996). Additions of vitamin D_3 or RA during these cultures fail to inhibit the functional dedifferentiation as well as the reduced CaR and megalin mRNA levels (Nygren et al., 1988). However, culturing of pathological, already functionally dedifferentiated, human parathyroid cells, demonstrates maintenance of their degree of calcium sensitivity for several weeks (Hellman et al., 1998).

While functional dedifferentiation is a characteristic disturbance of pathological parathyroid cells, the proliferative disturbance is another. Although rarely encountered in the clinical situation, with the possible exception of early stages of renal insufficiency, hypocalcemia has been demonstrated to stimulate proliferation of parathyroid cells in culture (Kremer et al., 1989; Naveh-Many et al., 1995). Active vitamin D_3 has effects on the parathyroid cell proliferation, possibly via inhibition of c-myc expression, which indicates that this transcription factor is important for the regulation of parathyroid cell growth (Kremer et al., 1989). Deficient production of active vitamin D is crucial for the development and progression of secondary HPT in uremia and may also significantly contribute to the pathogenesis of primary HPT in elderly individuals (Martin and Slatopolsky, 1994). HPT is also the most common disturbance seen in multiple endocrine neoplasia type 1 (MEN-1), but this variant of HPT demonstrates a different pathophysiological pattern, characterized mainly by an increased proliferation rate and less functional disturbance. This combination is reflected in a close to normal set-point for the $[Ca^{2+}]_i$ regulation (and PTH release) and comparably high expression of CaR and megalin (Carling et al., 1995).

Genetic disturbances

The most likely important reason for the increased cell proliferation of parathyroid adenomas is various genetic disturbances, which may be familial inherited or acquired. Familial HPT may be associated with MEN-1 or occur in kindreds not suffering from MEN-1. The MEN-1 gene on chromosome 11q13 has recently been identified and found to encode a putative transcription factor *menin*, apparently acting as a suppressor of JunD-induced transcription (Chandrasekharappa et al., 1997; Agarwal et al., 1999). In non-MEN1 familial HPT, a tumor suppressor gene on chromosome 1q has been identified (Szabo et al., 1995), although there may apparently be other derangements in other families.

About 30% of sporadic parathyroid adenomas are associated with increased expression of cyclin D1, inducing the cells to leave the G1 phase to enter S phase (Arnold, 1994). This overexpression may result from an inversion on chromosome 11 causing the PTH promoter to be placed in front of the cyclin D1 gene (Arnold, 1994). At present there are no other types of discovered gene activation in sporadic parathyroid tumors, while loss of gene function has been demonstrated both by allelic losses and inactivating point mutations. Thus, allelic losses and mutations in the MEN1 gene also commonly occur in sporadic HPT (Carling et al., 1998; Farnebo et al., 1998). In addition, clonal allelic losses of loci on chromosome 1p has been observed in a substantial subset of parathyroid adenomas (Cryns et al., 1995).

Certain polymorphisms within the vitamin D receptor (VDR) gene were initially demonstrated to couple to the development of osteoporosis (Morrison et al., 1994). Although further analyses failed to support this hypothesis, the same polymorphisms have been associated with increased risk for development of primary HPT in postmenopausal women (Carling et al., 1995). Thus, postmenopausal women with the haplotype *ba*T express reduced VDR mRNA and probably protein

levels, with an expected reduction in inhibition of the transcription of several genes regulated by vitamin D (Carling et al., 1998). Concomitantly right-shifted set-points for the PTH release occur in pathological parathyroid glands from the individuals with the *ba*T haplotype when compared to those with other VDR haplotypes (Carling et al., 1997). In the older population there is an increased incidence of nephrosclerosis, giving rise to mainly subclinical reduction of the vitamin D levels. This may, at least in the North European countries, be aggravated by seasonal lack of sunshine, and cause insufficient inhibition of the vitamin D-related gene transcription (e.g. PTH and c-myc; Fig. 1). Interestingly, one of the genes that at least in some tissues is upregulated by vitamin D is VDR itself, whereby insufficient circulating vitamin D levels may aggravate the disturbance.

Histopathology

Parathyroid glands consist of chief and oxyphil cells, a fibrous stroma, and fat cells. The number of oxyphil cells increases with age, and although they may maintain some secretory function their role remains obscure. Fat may be stored not only in fat cells, but also as lipid droplets of variable size within the parenchymal chief cells. The total amount of fat is related to the activity of the gland and may thus relate to levels of ambient Ca^{2+}. A normal parathyroid gland (mean weight approx. 60 mg) consists on average of 50% fat, while a functionally active and proliferating adenoma may be more or less totally depleted of fat. Oil red O staining for visualization of cytoplasmic fat in parathyroid chief cells is an important histopathological method which may be used to distinguish normal and pathological glands (Grimelius et al., 1986). The functional parenchymal chief cells of normal parathyroid glands abundantly express both CaR and megalin.

The size of parathyroid adenomas varies considerably from that just exceeding a normal gland to several grams. A rim of normal parenchyma situated at one end of the pathological gland may often be visualized, representing its origin in normal glandular tissue, and is often used to substantiate the adenoma diagnosis. The pathological part of the gland expresses low amounts of the calcium-sensing proteins, both CaR and megalin, substantiated both at mRNA and protein levels, whereas the rim seems to exhibit high expression comparable with the normal parathyroid glands (Juhlin et al., 1988; Kifor et al., 1996; Lundgren et al., 1997). X-chromosome inactivation analyses of sporadic adenomas have revealed that these are monoclonal lesions, supporting the theory of development as a result of a specific genetic hit (Arnold et al., 1988).

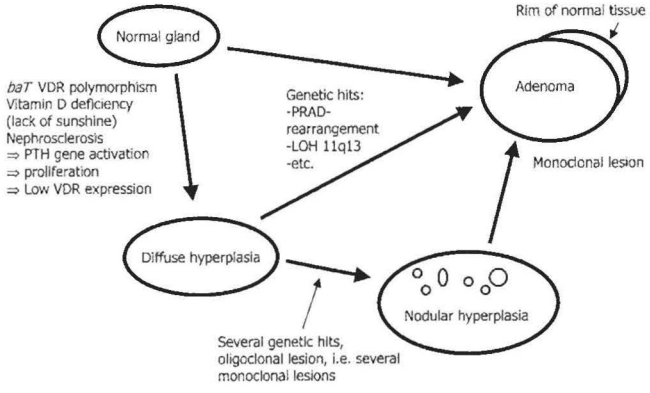

Fig. 1. Schematic drawing of suggested development of parathyroid hyperplasia and adenoma in postmenopausal women.

Primary parathyroid hyperplasia may be categorized as diffuse hyperplasia exhibiting a variable degree of an increased number of chief cells in all four parathyroid glands, or as nodular hyperplasia with irregularly distributed areas of apparently clonally expanding nodules of chief cells often surrounded by more normal-appearing tissue. The slowly proliferating diffuse hyperplasia appears to be a polyclonal lesion, both in sporadic (Arnold et al., 1988) and in familial HPT (Friedman et al., 1989), whereas the nodular hyperplasia of both sporadic and MEN-1-associated HPT has been proposed to arise from a background of polyclonal, diffuse hyperplasia, and to consist of several monoclonal lesions (Friedman et al., 1989; Arnold et al., 1995). The prevailing theory is that the diffusely hyperplastic tissue is more vulnerable to genetic hits, which favour the development of nodular hyperplasia after appearance of different secondary genetic hits (Fig. 1) (Åkerström et al., 1986; Arnold, 1994). Although beyond the scope of this review, there are many similar features between this type of primary hyperplasia and secondary HPT. Mild renal insufficiency consequently is characterized by a diffuse hyperplasia, whereas with increased duration and severity of the renal impairment a nodular growth pattern emerges within the glands (Åkerström et al., 1986; Wallfelt et al., 1988a,b; Hellman et al., 1989; Tominaga et al., 1996). These parenchymal cell nodules, which may be conspicuously large and apparently represent several monoclonal lesions, may present features similar to adenomas. The term oligoclonal lesion has been suggested for this disease (Fig. 1). More or less impaired renal function within a nephrosclerotic, elderly population may, especially in individuals harboring the *baT* haplotype of the VDR gene, constitute a similar prerequisite for the development of a diffuse primary hyperplasia, with the increased risk of emerging nodules, including possibly also large nodules mimicking adenomas (Fig. 1). In other terms, this abnormality in both "primary" and secondary HPT may possibly relate to genetic instability associated with impaired vitamin D action.

The calcium receptor expression in hyperplastic tissue varies. In sporadic hyperplasia different nodules within the same gland have been found to express strikingly different levels of megalin, which indeed supports the development of different monoclonal lesions (Juhlin et al., 1988). In general, the non-

nodulated tissue maintains higher expression than the nodules. Similar differences in distribution have been reported for CaR, and consequently deficient expression of calcium-sensing molecule(s) may constitute the necessary molecular pre-requisite for a right-shifted set-point seen in hyperplastic as well as adenomatous parathyroid tissue (Larsson et al., 1984; Juhlin et al., 1988; Farnebo et al., 1997).

The proliferation per se, however, could as a general feature of dedifferentiation result in reduced expression of several cell surface proteins, since other surface bound proteins also are altered in pathological parathyroid glands (Hellman et al., 1995a,b, 1996). However, the often highly variable expression of the same surface proteins within the same gland makes this explanation unlikely, and MEN-1, which is characterized by a substantial proliferative capacity, tends to exhibit almost normal expression of megalin as well as reasonably maintained Ca^{2+} regulation (Carling et al., 1995).

Treatment

The only definite treatment for primary HPT is surgery, but bisphosfonates and calcitonin have been used, especially in patients with severe hypercalcemia, e.g. hypercalcemic crisis (Grossman and Jossart, 1997). The operation of HPT aims to reduce the risk for consequences of untreated HPT. The classical ones, but nowadays seldom seen in Western countries, are bone disease (osteitis fibrosa cystica) and renal stones. However, recent studies have emphasized other signs and symptoms, such as mental disturbances like confusion, depression and dementia, muscle weakness and joint pain, and increased risk of cardiovascular diseases (Lundgren et al., 1998). Due to the development of sensitive methods to measure intact PTH in recent years, and reductions in peroperative complications, patients today tend to be operated at less extensive hypercalcemia. In addition, HPT is a common disorder, striking up to 2% of postmenopausal women, and parathyroid pathology can be found in 10% at post mortem studies (Åkerström et al., 1984). Altogether this accounts for a considerable number of patients, where the majority have mild HPT accompanied by mild or no overt symptoms and an increased long-term risk for cardiovascular diseases (Hedbäck et al., 1990). To treat this large population, several alternative medical treatments have been suggested. Estrogens have been discussed, but they exert comparably minor effects on the parathyroid, although are beneficial on bone loss. Vitamin D has a promising therapeutic potential, but also major side-effects, including hypercalcemia, hypercalciuria and soft tissue calcification caused by its intestinal and skeletal effects (Vieth, 1990). However, vitamin D analogues have been developed, which can inhibit PTH gene transcription and the parathyroid cell proliferation, but without the hypercalcemic effect. Retinoids or retinoid analogues with selective effects may also be used to attain these goals. A third novel alternative is usage of calcimimetics, which activate CaR and thereby inhibit PTH release (Nemeth et al., 1998).

The definite way of treatment, however, remains surgical. Skilled surgeons of today perform, with a minor degree of complications, various operations aiming at and achieving the goal of life-long normocalcemia in over 90% of the patients.

Acknowledgements. The financial support of the Swedish Medical Society, The Swedish Cancer Foundation, Selander's Foundation and the Swedish Medical Research Council are greatly acknowledged, as well as the critical reading of the manuscript by Prof. Jonas Rastad.

References

Agarwal S.K., Guru S.C., Heppner C., Erdos M.R., Collins R.M., Park S.Y., Saggar S., Chandrasekharappa S.C., Collins F.S., Spiegel A.M., Marx S.J. and Burns A.L. (1999). Menin interacts with the AP1 transcription factor JunD and represses JunD-activated transcription. Cell 96, 143-152.

Åkerström G., Malmaeus J., Grimelius L., Ljunghall S. and Bergström R. (1984). Histological changes in parathyroid glands in subclinical and clinical renal disease. Scand. J. Urol. Nephrol. 18, 75-81.

Åkerström G., Rudberg C., Grimelius L., Bergström R., Johansson H., Ljunghall S. and Rastad J. (1986). Histologic parathyroid abnormalities in an autopsy series. Hum. Pathol. 17, 520-527.

Arnold A. (1994). Molecular basis of primary hyperparathyroidism. In: The parathyroids. Levine M.A. and Bilezikian J.P. (eds). Raven Press, Ltd. New York, NY. pp 407-421.

Arnold A., Brown M., Urena P., Gaz R., Sarfati E. and Drueke T. (1995). Monoclonality of parathyroid tumors in chronic renal failure and in primary parathyroid hyperplasia. J. Clin. Invest. 95, 2047-2053.

Arnold A., Staunton C.E., Kim H.G., Gaz R.D. and Kronenberg H.M. (1988). Monoclonality and abnormal parathyroid hormone genes in parathyroid adenomas. N. Engl. J. Med. 318, 658-662.

Bai M., Trivedi S., Lane C.R., Yang Y., Quinn S.J. and Brown E.M. (1998). Protein kinase C phosphorylation of threonine at position 888 in Ca^{2+}o-sensing receptor (CaR) inhibits coupling to Ca^{2+} store release. J. Biol. Chem. 273, 21267-21275.

Baran D., Sorensen A., Shalboub V., Owem T., Oberdorf A., Stein G. and Lian J. (1991). 1α,25-dihydroxyvitamin D_3 rapidly increases cytosolic calcium in clonal rat osteosarcoma cells acking the vitamin D receptor. J. Bone Min. Res. 6, 1269-1275.

Bikle D.D., Ratnam A., Mauro T., Harris J. and Pillai S. (1996). Changes in calcium responsiveness and handling during keratinocyte differentiation. Potential role of the calcium receptor. J. Clin. Invest. 97, 1085-1093.

Bouillon R., Okamura W.H. and Norman A.W. (1995). Structure-Function relationships in the vitamin D endocrine system. Endocrine Rev. 16, 200-257.

Brown E. (1982). PTH secretion in vivo and in vitro. Regulation by calcium and other secretagogues. Miner Electrolyte Metab. 8, 130-150.

Brown E.M. (1983). Four-parameter model of the sigmoidal relationship between parathyroid hormone release and extracellular calcium concentration in normal and abnormal parathyroid tissue. J. Clin. Endocrinol. Metab. 56, 572-581.

Brown E.M. and Hebert S.C. (1996). Ca^{2+}-receptor-mediated regulation of parathyroid and renal function. Am. J. Med. Sci. 312, 99-109.

Brown E.M., Gamba G., Riccardi D., Lombardi M., Butters R., Kifor O., Sun A., Hediger M.A., Lytton J. and Hebert S.C. (1993). Cloning and characterization of an extracellular $Ca^{(2+)}$-sensing receptor from bovine parathyroid. Nature 366, 575-580.

Brown E.M., Gardner D.G., Brennan M.F., Marx S.J., Spiegel A.M., Attie M.F., Downs Jr R.W., Doppman J.L. and Aurbach G.D. (1979). Calcium-regulated parathyroid hormone release in primary hyperparathyroidism: Studies in vitro with dispersed parathyroid cells. Am. J. Med. 66, 923-931.

Cantley L.K., Russel J., Lettieri D. and Sherwood L.M. (1985). 1,25-dihydroxyvitamin D_3 suppresses parathyroid hormone secretion from bovine parathyroid cells in tissue culture. Endocrinology 117, 2114-2119.

Carlberg C. and Polly P. (1998). Gene regulation by vitamin D_3 [In Process Citation]. Crit. Rev. Eukaryot. Gene Expr. 8, 19-42.

Carling T., Correa P., Hessman O., Hedberg J., Skogseid B., Lindberg D., Rastad J., Westin G. and Akerstrom G. (1998). Parathyroid MEN1 gene mutations in relation to clinical characteristics of nonfamilial primary hyperparathyroidism [see comments]. J. Clin. Endocrinol. Metab. 83, 2960-2963.

Carling T., Kindmark A., Hellman P., Lundgren E., Ljunghall S., Rastad J., Åkerström G. and Melhus H. (1995). Vitamin D genotypes in hyperparathyroidism. Nature Med. 1, 1309-1311.

Carling T., Rastad J., Ridefelt P., Gobl A., Hellman P., Öberg K., Rask L., Larsson C., Juhlin C., Åkerström G. and Skogseid B. (1995). Hyperparathyroidism of multiple endocrine neoplasia type 1: Candidate gene and parathyroid calcium sensing protein expression. Surgery 118, 924-931.

Carling T., Rastad J., Åkerström G. and Westin G. (1998). Vitamin D receptor (VDR) and parathyroid hormone messenger ribonucleic acid levels correspond to polymorphic VDR alleles in human parathyroid tumors. J. Clin. Endocrinol. Metab. 83, 2255-2259.

Carling T., Ridefelt P., Hellman P., Rastad J. and Åkerström G. (1997). Vitamin D receptor polymorphisms correlate to parathyroid cell function in primary hyperparathyroidism. J. Clin. Endocrinol. Metab. 82, 1772-1775.

Chandrasekharappa S.C., Guru S.C., Manickam P., Olufemi S.-E., Collins F.S., Emmert-Buck M.R., Debelenko L.V., Zhuang Z., Lubensky I.A., Liotta L.A., Crabtree J.S., Wang Y., Roe B.A., Weisemann J., Boguski M.S., Agarwal S.K., Kester M.B., Kim Y.S., Heppner C., Dong Q., Spiegel A.M., Burns A.L. and Marx S.J. (1997). Positional cloning of the gene for multiple endocrine neoplasia-type 1. Science 276, 404-407.

Cryns V.L., Yi S.M., Tahara H., Gaz R.D. and Arnold A. (1995). Frequent loss of chromosome 1p DNA in parathyroid adenomas. Gene. Chromosome Cancer 13, 9-17.

Delmez J.A., Tindira C., Grooms P., Dusso A., Windus D.W. and Slatopolsky E. (1989). Parathyroid hormone suppression by intravenous 1,25-dihydroxyvitamin D. A role for increased sensitivity to calcium. J. Clin. Invest. 83, 1349-1355.

Demay M.B., Kiernan M.S., DeLuca H.F. and Kronenberg H.M. (1992). Sequences in the human parathyroid hormone gene that bind the 1,25-dihydroxyvitamin D_3 receptor and mediate transcriptional repression in response to 1,25-dihydroxyvitamin D_3. Proc. Natl. Acad. Sci. USA 89, 8097-8101.

Epstein P., Prentki M. and Attie M. (1985). Modulation of intracellular Ca^{2+} in the parathyroid cell. Release of Ca^{2+} from non-mitochondrial pools by inositol trisphosphate. FEBS Lett. 188, 141-144.

Farnebo F., Enberg U., Grimelius L., Bäckdahl M., Schalling M., Larsson C. and Farnebo L.-O. (1997). Tumor specific decreased expression of calcium sensing receptor message ribonucleic acid in sporadic primary hyperparathyroidism. J. Clin. Endocrinol. Metab. 82, 3481-3486.

Farnebo F., Teh B.T., Kytölä S., Svensson A., Phelan C., Sandelin K., Thompson N.W., Höög A., Weber G., Farnebo L.-O. and Larsson C. (1998). Alterations of the MEN 1 gene in sporadic parathyroid tumors. J. Clin. Endocrinol. Metab. 83, 2627-2630.

Farquhar M.G., Kerjaschki D., Lundstrom M. and Orlando R.A. (1994). Gp330 and RAP: The heymann nephritis antigenic complex. Ann. NY Acad. Sci. 737, 96-113.

Friedman E., Sakaguchi K., Bale A., Falchetti A., Streeten E., Zimering M., Weinstein L., McBride W., Nakamura Y. and Brandi M. (1989). Clonality of parathyroid tumors in familial multiple endocrine neoplasia type 1. N. Engl. J. Med. 321, 213-218.

Fujimi T., Baba H., Fukase M. and Fujita T. (1991). Direct inhibitory effect of aminoterminal parathyroid hormone fragment [PTH(1-34)] on PTH secretion from bovine parathyroid primary cultured cells in vitro. Biochem. Biophys. Res. Commun. 178, 953-958.

Garrett J.E., Tamir H., Kifor O., Simin R.T., Rogers K.V., Mithal A., Gagel R.F. and Brown E.M. (1995). Calcitonin-secreting cells of the thyroid express an extracellular calcium receptor gene. Endocrinology 136, 5202-5211.

Grimelius L., Åkerström G., Johansson H., Ljunghall S. and Rastad J. (1986). The role of pathology in diagnosis and surgical decision-making. In: Progress in surgery. Eh H. (ed). S. Karger. Basel. pp 80-92.

Grossman R.F. and Jossart G.H. (1997). Hypercalcemic crisis. In: Textbook of endocrine surgery. Clark O.H. and Duh Q.-Y. (eds). W.B. Saunders Company. Philadelphia, PA. pp 432-438.

Gylfe E., Johansson H., Larsson R., Nygren P., Rastad J., Wallfelt C. and Åkerström G. (1987). Activation of Ca^{2+} influx into parathyroid cells by external cation binding. In: Calcium regulation and bone metabolism: Basic and clinical aspects. Cohn D.V., Martin T.J. and Meunier P.J. (eds). Elsevier. Amsterdam. pp 33-38.

Gylfe E., Larsson R., Johansson H., Nygren P., Rastad J., Wallfelt C. and Åkerström G. (1986). Calcium-activated calcium permeability in parathyroid cells. FEBS Lett. 205, 132-136.

Habener J.F., Rosenblatt M. and Potts Jr J.T. (1984). Parathyroid hormone: Biochemical aspects of biosynthesis, secretion, action and metabolism. Physiol. Rev. 64, 985-1053.

Hedbäck G., Tisell L.-E., Bengtsson B.-Å., Hedman I. and Oden A. (1990). Premature death in patients operated on for primary hyperparathyroidism. World J. Surg. 14, 829-835.

Hellman P., Åkerström G., Ljunghall S. and Rastad J. (1989). Surgical findings and results of subtotal and total parathyroidectomy in hypercalcemic patients with uremic hyperparathyroidism. Acta Chir. Scand. 155, 573-582.

Hellman P., Ridefelt P., Juhlin C., Åkerström G., Rastad J. and Gylfe E. (1992). Parathyroid-like regulation of parathyroid hormone-related protein release and cytoplasmic calcium in cytotrophoblast cells of human placenta. Arch. Biochem. Biophys 293, 174-180.

Hellman P., Åkerström G., Juhlin C., Ridefelt P. and Rastad J. (1994). Pathophysiology of hyperparathyroidism. In: Current controversy in parathyroid operation and reoperation. Åkerström G., Juhlin C. and Rastad J. (eds). R.G. Landes Company. Austin, TX, USA. pp 9-22.

Hellman P., Juhlin C., Karlsson-Parra A., Klareskog L., Ridefelt P.,

Rastad J. and Åkerström G. (1995a). Expression and function of a CD_3-like molecule on normal and abnormal human parathyroid cells. Surgery 118, 1055-1062.

Hellman P., Ridefelt P., Björklund E., Yu W., Liu W., Tang S.-S., Juhlin C., Rastad J., Gylfe E., Lundgren S., Rask L., Ingelfinger J. and Åkerström G. (1995b). Cation sensing in a transformed rat proximal tubule cell line. Bone 16, S139.

Hellman P., Juhlin C., Karlsson-Parra A., Klareskog L., Lundgren S., Ridefelt P., Rastad J. and Åkerström G. (1996). Expression and function of a CD4-like molecule in normal and abnormal parathyroid cells - Relation to a calcium sensor molecule. Surgery 120, 985-992.

Hellman P., Liu W., Törmä H. and Åkerström G. (1998). Parathyroid cells are targets for retinoids. Bone 23, S452.

Hilpert J., Nykjaer A., Jacobsen C., Wallukat G., Nielsen R., Moestrup S.K., Haller H., Luft F.C., Christensen E.I. and Willnow T.E. (1999). Megalin antagonizes activation of the parathyroid hormone receptor. J. Biol. Chem. 274, 5620-5625.

Hjälm G., Murray E., Crumley G., Harazim W., Lundgren S., Onyango I., Ek B., Larsson M., Juhlin C., Hellman P., Davis H., Åkerström G., Rask L. and Morse B. (1996). Cloning and sequencing of human gp330, a Ca^{2+} binding receptor with potential intracellular signalling properties. Eur. J. Biochem. 239, 132-137.

Juhlin C., Holmdahl R., Johansson H., Rastad J., Åkerström G. and Klareskog L. (1987a). Monoclonal antibodies with exclusive reactivity against parathyroid cells and tubule cells of the kidney. Proc. Natl. Acad. Sci. USA 84, 2990-2994.

Juhlin C., Johansson H., Holmdahl R., Gylfe E., Larsson R., Rastad J., Åkerström G. and Klareskog L. (1987b). Monoclonal antiparathyroid antibodies interfering with a Ca^{2+}-sensor of human parathyroid cells. Biochem. Biophys. Res. Commun. 143, 570-574.

Juhlin C., Klareskog L., Nygren P., Ljunghall S., Gylfe E., Rastad J. and Åkerström G. (1988). Hyperparathyroidism is associated with reduced expression of a parathyroid calcium receptor mechanism defined by monoclonal antiparathyroid antibodies. Endocrinology 122, 2999-3001.

Juhlin C., Lundgren S., Johansson H., Lorentzen J., Rask L., Larsson E., Rastad J., Åkerström G. and Klareskog L. (1990). 500-kilodalton calcium sensor regulating cytoplasmic Ca^{2+} in cytotrophoblast cells of human placenta. J. Biol. Chem. 265, 8275-8279.

Jüppner H., Abou-Samra A.-B., Freeman M., Kong X.F., Schipani E., Richards J., Kolakowski Jr L.F., Hock J., Potts Jr J.T., Kronenberg H.M. and Segre G.V. (1991). A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide. Science 254, 1024-1026.

Kifor O., Moore F.D., Wang P., Goldstein M., Vassilev P., Kifor I., Hebert S.C. and Brown E.M. (1996). Reduced immunostaining for the extracellular Ca^{2+}-sensing receptor in primary and uremic secondary hyperparathyroidism. J. Clin. Endocrinol. Metab. 81, 1598-1606.

Kifor O., Diaz R., Butters R. and Brown E. (1997). The Ca^{2+}-sensing receptor (CaR) activates phospholipases C, A2, and D in bovine parathyroid and CaR-transfected, human embryonic kidney (HEK293) cells. J. Bone Miner. Res. 12, 715-725.

Kremer R., Bolivar I., Goltzman D. and Hendy G.N. (1989). Influence of calcium and 1,25-dihydroxycholecalciferol on proliferation and proto-oncogene expression in primary cultures of bovine parathyroid cells. Endocrinology 125, 935-941.

Larsson R., Wallfelt C., Abrahamsson H., Gylfe E., Ljunghall S., Rastad J., Rorsman P., Wide L. and Åkerström G. (1984). Defective regulation of the cytosolic Ca^{2+} activity in parathyroid cells from patients with hyperparathyroidism. Bioscience Rep. 4, 909-915.

Liu W., Hellman P., Li Q., Yu W.-R., Juhlin C., Nordlinder H., Rollman O., Åkerström G., Törmä H. and Melhus H. (1996). Biosynthesis and function of all-trans- and 9-cis-retinoic acid in parathyroid cells. Biochem. Biophys. Res. Commun. 229, 922-929.

Lopez-Barneo J. and Armstrong C.M. (1983). Depolarizing response of rat parathyroid cells to divalent cations. J. Gen. Physiol. 82, 269-294.

Lundgren E., Ljunghall S., Åkerström G., Hetta J., Mallmin H. and Rastad J. (1998). Case-control study on symptoms and signs of "asymptomatic" primary hyperparathyroidism. Surgery 124, 980-986.

Lundgren S., Carling T., Hjälm G., Juhlin C., Rastad J., Pihlgren U., Rask L., Åkerström G. and Hellman P. (1997). Tissue distribution of human gp330/megalin, expressing putative Ca^{2+} sensing properties the 550 kDa calcium sensor protein. J. Histochem. Cytochem. 45, 383-392.

Lundgren S., Hjälm G., Hellman P., Ek B., Juhlin C., Rastad J., Klareskog L., Åkerström G. and Rask L. (1994). A protein involved in calcium sensing of the human parathyroid and placental cytotrophoblast cells belonging to the LDL-receptor superfamily. Exp. Cell Res. 212, 344-350.

MacDonald P.N., Ritter C., Brown A.J. and Slatoposlky E. (1994). Retinoic acid suppresses parathyroid hormone (PTH) secretion and preproPTH mRNA levels in bovine parathyroid cell culture. J. Clin. Invest. 93, 725-730.

Martin K.J. and Slatopolsky E. (1994). The parathyroids in renal disease. Pathophysiology. In: The parathyroids. Bilezekian J.P., Levina M.A. and Marcus R. (eds). Raven Press Ltd. New York. pp 711-719.

McKay C. and Miller A. (1996). Relationship among cellular diacylglycerol, sphingosine formation, protein kinase C activity, and parathyroid hormone secretion from dispersed bovine parathyroid cells. Endocrinology 137, 2473-2479.

Morrison N.A., Cheng Qi J., Tokita A., Kelly P.J., Crofts L., Nguyen T.V., Sambrook P.N. and Eisman J.A. (1994). Prediction of bone density from vitamin D receptor alleles. Nature 367, 284-287.

Naveh-Many T., Rahamimov R., Livni N. and Silver J. (1995). Parathyroid cell proliferation in normal and chronic renal failure rats. The effect of calcium, phosphate and vitamin D. J. Clin. Invest. 96, 1786-1793.

Nemeth E.F. and Scarpa A. (1986). Cytosolic Ca^{2+} and the regulation of secretion in parathyroid cells. FEBS Lett. 203, 15-19.

Nemeth E., Steffey M.E., Hammerland L.G., Hung B.C., van Wagenen B.C., DelMAr E.G. and Balandrin M.F. (1998). Calcimimetics with potent and selective activity on the parathyroid calcium receptor. Proc. Natl. Acad. Sci. USA 95, 4040-4045.

Nygren P., Larsson R., Johansson H., Ljunghall S., Rastad J. and Åkerström G. (1988). $1,25(OH)2D_3$ inhibits hormone secretion and proliferation but not functional dedifferentiation of cultured bovine parathyroid cells. Calcif. Tissue Int. 43, 213-218.

Nykjaer A., Dragun D., Walther D., Vorum H., Jacobsen C., Herz J., Melsen F., Christensen E.I. and Willnow T.E. (1999). An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D_3. Cell 96, 507-15.

Okazaki T., Ando K., Igarshi T., Ogata E. and Fujita T. (1992). Conserved mechanism of negative gene regulation by extracellular calcium. J. Clin. Invest. 89, 1268-1273.

Pollak M.R., Brown E.M., Wu Chou Y.-H., Hebert S.C., Marx S.J., Steinmann B., Levi T., Seidman C.E. and Seidman J.G. (1993).

Mutations in the human Ca^{2+}-sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Cell 75, 1297-1303.

Pollak M.R., Wu Chou Y.-H., Marx S.J., Steinmann B., Cole D.E.C., Brandi M.L., Papapoulos S.E., Menko F.H., Hendy G.N., Brown E.M., Seidman C.E. and Seidman J.G. (1994). Familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Effects of mutant gene dosage on phenotype. J. Clin. Invest. 93, 1108-1112.

Racke F.K. and Nemeth E.F. (1993). Protein kinase C modulates hormone secretion regulated by extracellular polycations in bovine parathyroid cells. J. Physiol. 468, 163-176.

Ridefelt P., Hellman P., Wallfelt C., Åkerström G., Rastad J. and Gylfe E. (1992a). Neomycin interacts with Ca^{2+} sensing of normal and adenomatous parathyroid cells. Mol. Cell. Endocrinol. 83, 211-218.

Ridefelt P., Nygren P., Hellman P., Larsson R., Rastad J., Åkerström G. and Gylfe E. (1992b). Regulation of parathyroid hormone release in normal and pathological parathyroid cells exposed to modulators of protein kinase C. Acta Endocrinol. 126, 505-509.

Ridefelt P., Björklund E., Åkerström G., Olsson M.J., Rastad J. and Gylfe E. (1995). Ca^{2+}-induced Ca^{2+} oscillations in parathyroid cells. Biochem. Biophys. Res. Commun. 215, 903-909.

Ridefelt P., Hellman P., Rastad J., Larsson R., Åkerström G. and Gylfe E. (1996). Effects of calcium channel modulators on the regulation of cytoplasmic Ca^{2+} and hormone secretion of parathyroid cells. Pharmacol. Toxicol. 78, 147-153.

Ruat M., Molliver M.E., Snowman A.M. and Snyder S.H. (1995). Calcium sensing receptor: molecular cloning in rat and localization to nerve terminals. Proc. Natl. Acad. Sci. USA 92, 3161-3165.

Saito A., Pietromonaco S., Kwor-Chieh Loo A. and Farquhar M.G. (1994). Complete cloning and sequencing of rat gp330/"megalin", a distinctive member of the low density lipoprotein receptor gene family. Proc. Natl. Acad. Sci. USA 91, 9725-9731.

Shivji F., Cheng H., Zwiers H., Hollenberg H. and Hanley D. (1996). Identification of classical, novel, and atypical protein kinase C isoenzymes in the bovine parathyroid. Endocrinology 137, 3777-3783.

Shoback D., Membreno L. and McGhee J. (1988). High calcium and other divalent cations increase inositol triphosphate in bovine parathyroid cells. Endocrinology 123, 283-289.

Silver J., Russell J. and Sherwood L.M. (1985). Regulation by vitamin D metabolites of messenger ribonucleic acid for preproparathyroid hormone in isolated bovine parathyroid cells. Proc. Natl. Acad. Sci. USA 82, 4270-4273.

Szabo J., Heath B., Hill V.M., Lackson C.E., Zarbo R.J., Mallette L.E., Chew S.L., Besser G.M., Thakker R.V. and Huff V. (1995). Hereditary hyperparathyroidism-jaw tumor syndrome: the endocrine tumor gene HRPT2 maps to chromosome 1q21-q31. Am. J. Hum. Genet. 56, 944-950.

Tang S.-S., Jung F., Diamant D., Brown D., Bachinsky D., Hellman P. and Ingelfinger J. (1995). Temperature-sensitive SV40 immortalized rat proximal tubule cell line has functional renin-angiotensin system. Am. J. Physiol. 268, F435-F446.

Tominaga Y., Kohara S., Namii Y., Nagasaka T., Haba T., Uchida K., Numano M., Tanaka Y. and Takagi H. (1996). Clonal analysis of nodular parathyroid hyperplasia in renal hyperparathyroidism. World J. Surg. 20, 744-752.

Wallfelt C., Gylfe E., Larsson R., Ljunghall S., Rastad J. and Åkerström G. (1988a). Relationship between external and cytoplasmic calcium concentrations, parathyroid hormone release and weight of parathyroid glands in human hyperparathyroidism. J. Endocrinol. 116, 457-464.

Wallfelt C., Larsson R., Gylfe E., Ljunghall S., Rastad J. and Åkerström G. (1988b). Secretory disturbance in hyperplastic parathyroid nodules of uremic hyperparathyroidism: Implications for parathyroid autotransplantation. World J. Surg. 12, 431-436.

Vieth R. (1990). The mechanisms of vitamin D toxicity. Bone Miner. 11, 267-272.

Willnow T., Hilpert J., Armstrong S., Rohlmann A., Hammer R., Burns D. and Herz J. (1996). Defective forebrain development in mice lacking gp330/megalin. Proc. Natl. Acad. Sci. USA 93, 8460-8464.

Accepted November 18, 1999