

# STARLING REVIEW

## Parathyroid hormone: past and present

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### Abstract

Research on parathyroid hormone (PTH) has undergone four rather distinctive phases, beginning just before the turn of the 20th century. Early debates about the function of the parathyroids were resolved by 1925, when understanding the role of PTH led to comprehending the action of the glands in calcium physiology. Elucidation of the pathophysiology of hormone excess (severe bone loss) and deficiency (hypocalcemia) continued over the following decades. With the advent of advances in chemical and molecular biology, the structure of PTH and its principal

receptor (PTHrP-receptor [PTHr1]) were established. Tests with purified hormonal peptide in humans led to the surprising, even paradoxical, finding that PTH can be used pharmacologically to build bone, providing a dramatic therapeutic impact on osteoporosis. These developments have stimulated the field of calcium and bone biology and posed new questions about the role of PTH as well as possible new directions in therapy.

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### History

Discovery of the parathyroid glands and their biological role evolved later than that of the thyroid gland (from which, somewhat ignominiously, the parathyroids derived their name). Four rather distinctive phases of study can be described over the last hundred years. These include determination of the physiological function of the parathyroids, the pathophysiology due to hormone excess or deficiency, chemical characterization and synthesis of parathyroid hormone (PTH) and study of its molecular and cellular biology, and, finally, the pharmacological use of PTH as an effective treatment for osteoporosis – a use that seems paradoxical in light of the hormone's physiological role and pathophysiological effects (See Table 1).

The glands were discovered in the late 19th century, and interest in their function increased during the first 25 years of the 20th century. An early excellent review (Boothby 1921) outlines the scientific issues and controversies during this period. There was concurrence that removal of all of the parathyroid glands in cats and dogs, as well as rodents, was associated with death from tetany (Gley 1891). It was agreed that the function of the parathyroid glands was distinct and separate from that of the thyroid, their only relationship being anatomic and not functional. It was also known that preservation of small amounts of parathyroid gland tissue or autotransplantation

of a single parathyroid gland would protect against tetany when the remaining parathyroids were deliberately removed. It was also recognized that patients undergoing extensive thyroid surgery occasionally suffered from tetany, presumably due to inadvertent removal of the parathyroid glands by the surgery. A decade after Gley's studies, the great pathologist, Jacob Erdheim, corroborated Gley's findings through meticulous research involving careful examination of tissue in the neck of animals in which tetany developed (Erdheim 1904). Erdheim established that no parathyroid tissue remained in those with tetany. He showed at postmortem that the same was true for patients dying from postoperative tetany after thyroid surgery (determined by painstaking histological examination of all tissue in the neck and finding that no parathyroid glands remained) (Erdheim 1906). He therefore affirmed the view that the cause of the tetany was the loss of the parathyroid tissue. Boothby (1921) concluded 'No one has recently questioned the importance of the parathyroids or the fact that their complete extravation usually results in tetany followed by death'.

Intense debate, which may seem surprising from our present perspective, centered around the cause of the tetany. The true explanation, severe hypocalcemia, was documented by a number of investigators (Boothby 1921). However, other investigators concluded that the principal function of the parathyroid glands was detoxification.

**Table 1** Chronology of major advances in PTH research

Date	
1850–1900	Parathyroid glands discovered as separate entities from thyroid Function unknown
1900–1925	Parathyroid gland function debated Tetany after parathyroidectomy: cause—hypocalcemia vs methyl guanidine
1925	Active gland extract purified Calcium regulation established
1927–1950s	Pathophysiology of hormone excess and deficiency defined Hyper- and hypoparathyroidism
1929	Bone mass increase in rats Paradox (largely ignored)
1970s	Hormone structure and synthesis Bone anabolic effects in animals confirmed Human clinical trials in osteoporosis start
1990s	Parathyroid hormone receptor cloned Rapid advances in understanding hormone action at the cellular and molecular level PTHrP gene knockout—abnormal bone development
2001	Striking clinical benefit in osteoporosis established Era of skeletal ‘anabolic’ agents begins

Strong proponents of the view that the function of the parathyroid glands was the control of blood calcium were MacCallum and various co-workers (MacCallum & Voegtlin 1908, MacCallum 1911, MacCallum & Vogel 1913). Among other findings, they demonstrated that infusion of calcium or administration of large doses of calcium lactate by mouth would prevent tetany. These findings led them to the conclusion that the normal purpose of the parathyroid glands was to control the blood calcium level. However, a problem arose in this line of reasoning: parenterally administered extracts of the parathyroid glands failed to reverse the tetany. This latter observation led others to conclude that the glands were primarily involved in detoxification. Koch (1912) found excessive levels of methyl guanidine in the urine of dogs and proposed that methyl guanidine caused the tetany. Others (Paton *et al.* 1914–1915) found that administration of guanidine and methyl guanidine in animals caused symptoms characteristic of tetany. The field remained hotly controversial, with the endocrine physiologist, Sharpey-Schäffer, declaring conclusively that the parathyroid glands did not produce any active secretion (Collip 1925).

Finally, a definitive series of experiments (Collip 1925) resolved the controversy and established the principal physiological role of the parathyroid glands in calcium regulation as we now understand it. Collip prepared hot hydrochloric acid extracts of the parathyroid glands, an approach which he correctly deduced was needed to free the active substance from other gland stromal components and render it soluble. He showed that these acid extracts of the parathyroid gland would completely relieve the tetany that followed parathyroidectomy, and established the parathyroids as an endocrine gland which secreted hormone

(PTH). Hanson (1924) claimed priority for this acid extraction procedure. However, as will be noted later, the approach, while successful, caused other difficulties when attempts were made to purify and characterize the active principle of the glands. This matter was not resolved for another 35 years, until the work of Aurbach (1959).

Pathophysiology

The second phase of work on PTH focused on understanding, subsequent to Collip’s confirmation of the endocrine status of the parathyroid glands, the harmful results of excess and/or insufficient PTH. This pioneering work by clinical investigators should be appreciated in terms of the limited tools available throughout much of the first half of the 20th century. X-rays were not available at the turn of the century, and measurements of mineral ions were tedious and frequently inexact. Metabolic studies could be performed only in a few research-oriented institutions in the early decades of the century. Communication among medical centers about important findings occurred much more slowly than today; nonetheless, remarkable progress was made in defining the consequences of deficient parathyroid action in humans (hypoparathyroidism) and later, of excess PTH due to parathyroid tumor (hyperparathyroidism).

Hypoparathyroidism

The consequences of PTH deficiency had already been defined in animals, as noted above. Hypocalcemia, hyperphosphatemia and tetany were established as the invariant consequences of hypoparathyroidism in humans, a

disorder which was most commonly post-surgical. It was gradually recognized to occur also as an idiopathic disease, a disorder which, at the beginning of the 21st century, we now know through careful genetic studies can be attributed to a number of different genetic mutations (Thakker & Jüppner 2005).

It was found that PTH action was insufficiently short in duration to reverse adequately the hypocalcemic and hyperphosphatemic abnormalities in patients with hypoparathyroidism (calcium and vitamin D supplements were needed). In an elegant treatise (Albright & Reifenstein 1948), much of this early work on the pathophysiology of hyper- and hypoparathyroidism was well-documented and referenced.

An interesting example of the prescience of the great clinical investigators was the clear delineation of a form of hypoparathyroidism due to resistance to hormone action rather than defective hormone production. This entity, pseudohypoparathyroidism, was correctly interpreted by Albright and colleagues to be a form of hormone resistance. Administration of a PTH-containing extract to patients with pseudohypoparathyroidism (with their associated skeletal and other phenotypic features) produced no response, in striking contrast to the vigorous response seen in patients with true hypoparathyroidism (i.e. deficient hormone production). Even more stunning was the ability of these investigators a few years later to deduce that a second patient with a similar skeletal phenotype but without any apparent disorder in calcium and phosphate metabolism was a different form of the same hereditary defect. Albright and colleagues waggishly referred to the disorder in this second patient as 'pseudo-pseudohypoparathyroidism' (a disorder with the abnormal skeletal phenotype but without hypocalcemia). Only at the close of the 20th century was the genetic defect in pseudohypoparathyroidism defined. The defect is an inactivating mutation in one of the alleles of the  $G_{s\alpha}$  subunit of the heterotrimeric G protein, a necessary co-factor in hormone/receptor activation (Thakker & Jüppner 2005). Normally, in all individuals, there is silencing of the paternal allele in the renal cortex, areas important for the formation of  $1,25(\text{OH})_2\text{D}$  and the PTH-driven inhibition of phosphate reabsorption; only the maternal allele is expressed in the renal cortex. If the defective allele comes from the father, there is no metabolic defect – only a skeletal phenotype is evident; if it comes from the mother, the hypocalcemic-hyperphosphatemic disorder is apparent. The skeletal phenotype appears to be due to the requirement for bi-allelic expression of the G protein subunit during embryonic development.

As noted by Albright and Reifenstein in their 1948 review, different forms of hypoparathyroidism – including post-surgical, idiopathic, pseudohypoparathyroidism, and those associated with Addison's disease (part of the multiple endocrine deficiency syndrome) – were all apparent in 1948. Of course, extensive genetic investigations in

the last 20 years, abetted by the successful completion of the Human Genome Project, have led to genetic definitions that are precise and valuable in overall diagnosis and management; however, it is impressive to note the insights gained many decades earlier by precise clinical investigation.

### *Hyperparathyroidism*

The sequence of events that led to the definition of hyperparathyroidism as a clinical entity (reviewed in Albright & Reifenstein 1948, Albright & Ellsworth 1990) represented a coincidence of insights gained from the impressive studies of pathology of disease in Europe and mechanism-based studies in the emerging clinical research wards in a few medical centers in the US. The pioneering work of Virchow and, later, Erdheim (with whom Albright studied) had established not only that the total absence of the parathyroid glands led to severe hypocalcemia and tetany but also pointed to enlargement of the parathyroid glands in association with disease. The most notable findings, however, were in osteomalacia, where Erdheim observed that all four parathyroid glands were grossly enlarged. (From today's perspective, we would understand this as a compensation for vitamin D deficiency associated with osteomalacia – secondary hyperparathyroidism (Thakker & Jüppner 2005).

A second line of investigation in Europe regarding bone disease provided a new clue in recognizing hyperparathyroidism. As noted by Albright and Ellsworth (1990), the first report of the disease that is today called 'osteitis fibrosis cystica' was given at a Festschrift for Virchow in 1891 by von Recklinghausen (the cause was unknown). Although in both diseases the bones are demineralized, the two conditions could be distinguished by some clinicians and by pathologists based on postmortem findings because of the extensive cysts and greater incidence of fractures in osteitis fibrosa cystica. Some investigators had suggested that when only one parathyroid gland was enlarged, it might be the cause of osteitis fibrosa cystica rather than a secondary adaptation.

In 1924, a patient with severe demineralization and multiple fractures was evaluated in Vienna. X-ray studies were available and confirmed severe bone loss and multiple cysts. Because of persistent views that severe bone disease was likely due to a deficiency of PTH action (all four glands were enlarged, according to Erdheim), the first procedure tried in the patient was transplantation of parathyroid glands obtained from an accident victim. The transplantation was without benefit. Finally, in the summer of 1925, Mandl operated on the neck of the patient (where enlarged glands were typically found) and removed an enlarged parathyroid tumor. Dramatic improvement and total healing followed (Albright & Reifenstein 1948).

At about the same time in the US, Captain Charles Martell, a much-admired patient whose long illness was recorded in multiple publications (Albright & Reifstein 1948, Bauer & Federman 1962, Albright & Ellsworth 1990) was disabled with what, in retrospect, was hyperparathyroidism with severe osteitis fibrosis cystica. The first patient with the disease to be recognized in the US, Martell endured a much more painful and prolonged saga than did the Viennese patient. (Although it was not appreciated at the time due to lack of detailed knowledge about variation in location of the parathyroid glands, Martell did have a parathyroid adenoma; unfortunately, the tumor was located in the chest rather than, as the pathologists of the day understood, in the thyroid area of the neck).

His diagnosis was first indicated by detection of hypercalcemia by DuBois in New York City's Bellevue Hospital (Albright & Ellsworth 1990). Collip's work had shown that PTH extracts could even raise blood calcium if given in large amounts – hence, the suspicion of hyperparathyroidism arose. However, given the novelty of the patient's problem – no one else having been operated on for this disease, let alone cured – he was referred to Joseph Aub at the Massachusetts General Hospital in Boston. Aub had been using PTH, available by that time as a result of Collip's work, to mobilize lead from the bones of patients who had incurred lead poisoning (Albright & Ellsworth, 1990). He had studied parathyroid action and used metabolic balance techniques to determine the calcium input and output in patients. Martell's hypercalcemia was confirmed and a negative calcium balance shown. After several initial operations were unsuccessful, the patient was placed on a high calcium intake in an effort to replete the skeletal deficit. Although he was converted to a positive calcium balance with considerable healing of the bones, this occurred at the expense of worsening hypercalcemia, continued high urinary calcium output, and kidney stones. When a report eventually appeared regarding another patient in Europe who died from von Recklinghausen's disease and was found at postmortem to have a parathyroid adenoma in the mediastinum, the surgeons in Boston undertook yet another operation, this time successfully, with removal of the mediastinal parathyroid adenoma. Unfortunately, the patient died within a month due to renal complications from his long-standing bout with the disease. By this time, a number of patients with hyperparathyroidism had been cured by neck exploration in Europe and the US.

Many different features of hyperparathyroidism were described by several centers in Europe and the US during subsequent decades, and the classic features – such as bone disease, kidney stones, and a variety of systemic symptoms – were well described. Diagnosis needed to be made carefully in borderline cases using multiple metabolic tests, particularly in patients suffering from kidney stones but not the classic bone disease; diagnosis was carried out without the benefit of a specific test to measure

PTH in blood, a technique not developed until much later (a specific radioimmunoassay was reported in 1963 by Berson *et al.* (see below)).

As documented in clinical reviews in the late 20th century (Potts 1991, Bilezikian *et al.* 2002), the disease spectrum changed, with many patients lacking not only bone and kidney disease but any symptoms at all, thus leading to the term asymptomatic hyperparathyroidism. A full explanation as to why the disease spectrum has changed so dramatically is not apparent; but, clearly, a heightened awareness of the disease's presence through the uniform use of serum calcium measurements and the availability of highly selective and sensitive PTH immunoassays probably explains, in part, the detection of the disease in a milder form, as reviewed by Potts (1991) and Bilezikian *et al.* (2002).

### **Chemical characterization and synthesis of PTH: molecular and cellular biology of PTH action**

Although the field of PTH research, particularly the understanding of its clinical implications in disease, was greatly aided by Collip's breakthrough in preparing active extracts, progress was slow in determining its chemical structure. This occurred, in part, as an undesired side-effect of the successful hot acid procedure used by Collip. As we understand at present, the hormonal polypeptide was not only liberated and solubilized by hot acid extraction but also cleaved at multiple sites, particularly at sites within the molecule where aspartic acid or asparagine is found. The cleavage induced by dilute acid gives a multiplicity of biologically active products of varying chain length. In a 1954 report, Handler *et al.* summarized their frustration with efforts to purify the parathyroid polypeptide by stating '(1) the active material in the gland . . . may be a large protein which in the course of the isolation is degraded into fractions of varying size, each of which still has activity; (2) the active material may not be a large molecule at all, but instead a small molecule which adheres to each one of these fractions'. In other words, it seemed impossible to purify the hormone if it kept subdividing into multiple fractions, frustrating efforts to obtain a respectable yield of the material for chemical analysis. Their first conclusion (Handler *et al.* 1954) was the accurate one, but the active substance was already fractionated in the starting material, the hot acid extract. Aurbach (1959), and then Rasmussen and Craig (1959), solved the problem some 35 years following the original Collip observation. Considering the unwanted side-effects of hot acid extraction, they turned to extraction with organic solvents which accomplished the same task – namely, liberating the active principle (polypeptide) from the other cellular constituents without fragmenting it into several pieces. These breakthrough observations signaled the beginning of rapid chemical characterization and

synthesis. Two independent groups determined the structure of bovine and human PTH (and one of the groups that of porcine PTH) by conventional techniques of protein sequence analysis after laborious accumulation of sufficient material, particularly difficult with the human hormone available only from surgically removed tumors (Brewer & Ronan 1970, Niall *et al.* 1970, 1978, Brewer *et al.* 1972, Sauer *et al.* 1974, Keutmann *et al.* 1978). Based on the deduced amino acid sequence and the knowledge that hot acid produced active fragments, it was deduced that the first 34 amino-terminal amino acids should be sufficient for biological activity. The PTH(1–34) regions of first bovine and then human PTH were synthesized (Potts *et al.* 1971, Tregear *et al.* 1973, 1974). These fragments are analogous to the natural peptide fragments in the Collip preparation, but the latter were heterogeneous. The biological activities of the purified natural peptide and the synthetic amino terminal sequence of 34 residues were shown to be similar *in vitro* and *in vivo*.

Availability of highly purified parathyroid polypeptide and active synthetic fragments made it possible to develop radioimmunoassays and to open the era of molecular and cell biology in which the PTH actions at target sites could be properly evaluated and defined with purified hormone preparations. Of course, great advances in cell biology in other fields provided techniques that helped accelerate advances in PTH research. In addition, in the early 1970s, breakthroughs in molecular biology culminated in the development of recombinant DNA technology, which made it possible to deduce polypeptide sequence from nucleotide sequence of the responsible gene experimentally through analysis of cDNA – that is, a reverse copy of the mRNA for the protein. It became possible to deduce the amino acid sequence of the hormone in species in which the active hormonal polypeptide principle had never been isolated; the active peptide could then be synthesized.

### Structure/activity studies of the PTH ligand

Structures of mammalian forms of PTH and chicken PTH, shown in Fig. 1, also include the structures of PTH-related peptide (PTHrP). The two molecules share structural homology and some overlap in function (using the same G protein-linked receptor, discussed below). An extensive evaluation of PTHrP (Martin *et al.* 2005) is beyond the scope of this review but is included here for purposes of comparison. The structure of PTHrP, deduced late in the 20th century by nucleotide sequence analysis rather than the classic technique of protein isolation and structural determination (as was used for many of the PTH molecules), was triggered by the search for the cause of hypercalcemia of malignancy. There is clear evidence of a common ancestry for PTH and PTHrP that can be inferred from the similarity in their amino terminal sequence regions, the intron–exon organization of the

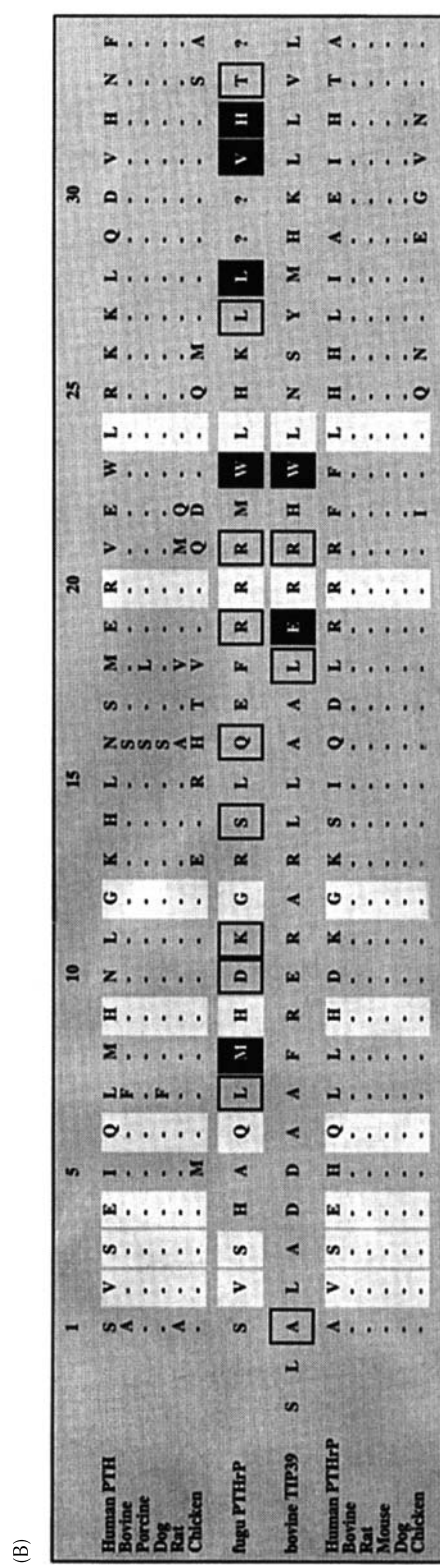
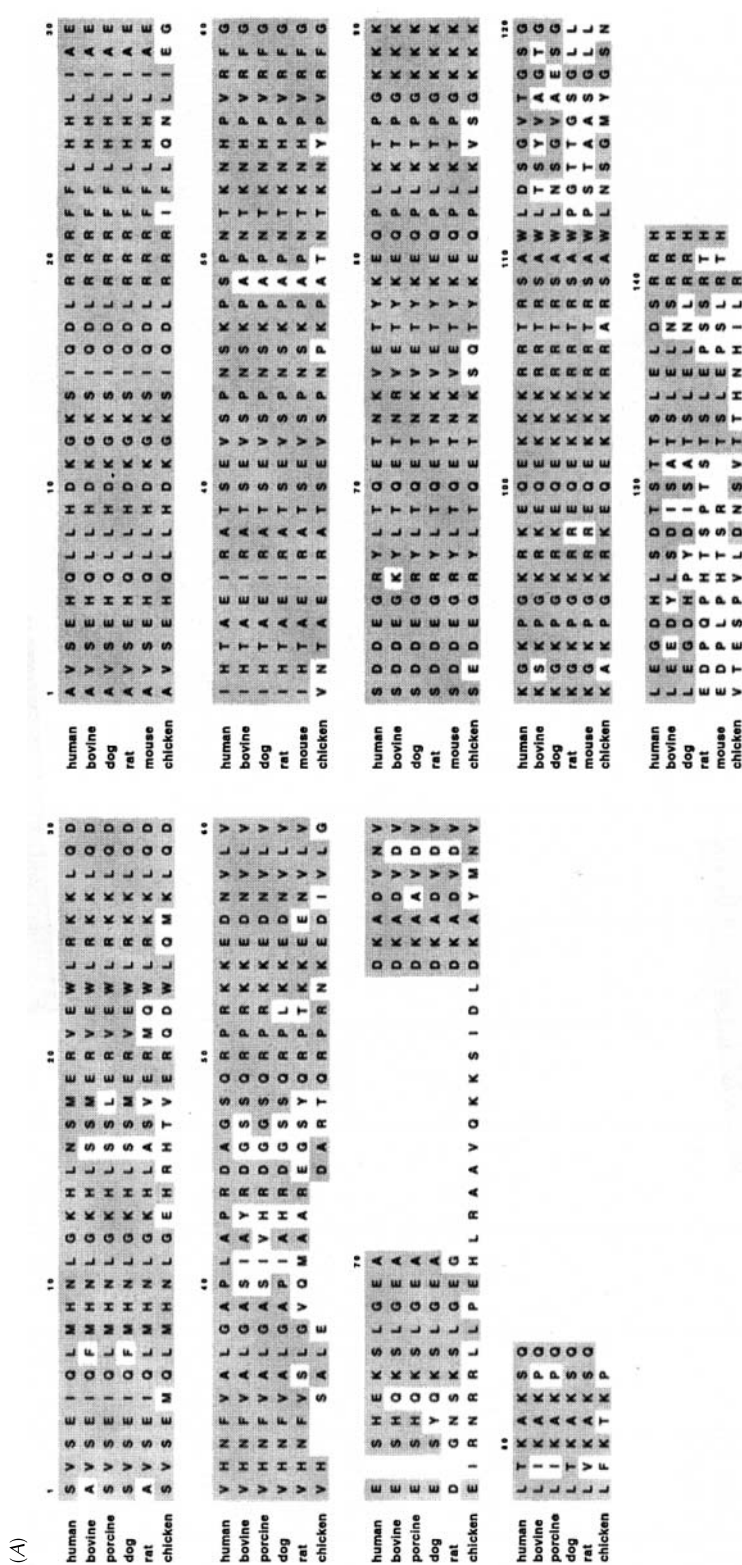
genes encoding the two molecules, and the structure of the exons that encode part of the precursor peptide (pro-peptide sequence). Both molecules have bone as a principal target tissue: PTHrP is vital during embryogenesis in regulating bone formation while PTH, possibly the later evolutionary arrival, has as its principal physiological function the mobilization of calcium from bone in the adult as part of its protection of calcium homeostasis (Jüppner *et al.* 2000).

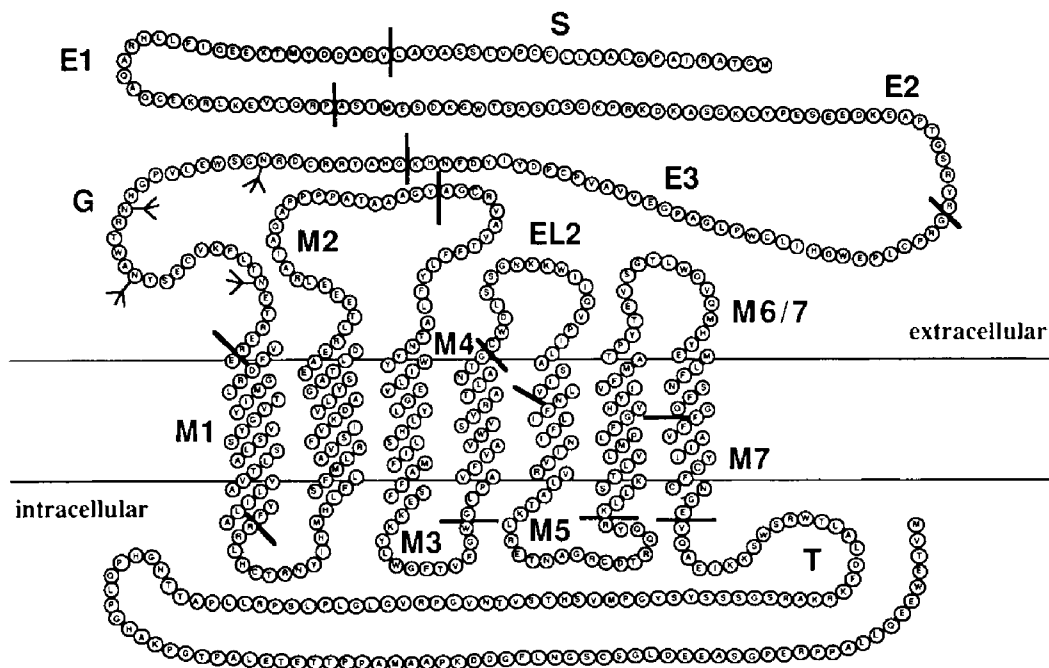
Nucleotide sequence analysis of cDNA for human and bovine PTH was used to confirm the amino acid sequences that had originally been determined by conventional peptide sequence analysis (Kronenberg *et al.* 1979, Hendy *et al.* 1981). The amino acid sequences of rat, chicken and dog PTH were determined exclusively by molecular cloning techniques without isolation of the protein (Heinrich *et al.* 1984, Khosla *et al.* 1988, Russell & Sherwood 1989, Jüppner *et al.* 2000).

Extensive sequence homology is present in the known mammalian PTH species (Fig. 1A, left panel). PTHrP structures are shown for comparison (Fig. 1A, right panel). All mammalian PTH molecules consist of a single chain polypeptide with 84 amino acids and a molecular weight of approximately 9400 Daltons. Chicken PTH, however, shows significant differences in comparison with the mammalian homologs; for example, avian PTH contains two deletions in the hydrophobic middle portion of the sequence and an additional 22 amino acids near the C-terminus, which replaces the stretch of nine amino acids, residues 62 to 70, in the mammalian hormones (Fig. 1A, left panel). As discussed above, the amino terminal region of PTH, which is the minimum sequence necessary and sufficient for regulation of mineral ion homeostasis, shows high sequence conservation among all vertebrate species and also, to a considerable extent, with PTHrP (Fig. 1B). The degree of sequence preservation in PTH molecules is less in the middle and carboxyl terminal regions than in the highly conserved amino terminal region (Fig. 1).

Parathyroid glands could not be identified in fish; however, PTH has been identified in fish, although the tissue of origin remains unclear since the identification and structure of PTH was carried out as part of the analysis of the complete genome of various fish species. PTH has been identified in catfish and also in fugu fish; two different PTH molecules have been seen in zebrafish. These fish molecules are shorter in overall length than mammalian PTH but, as seen in Fig. 1B, the fugu fish PTHrP retains much homology in the amino terminal portion of the molecule to the mammalian forms of PTH. Homology (not shown in Fig. 1) is also retained for the two forms of zebrafish PTH. The biological activity of several fish PTH molecules has been tested by synthesis of their amino terminal regions. These fish peptides do activate the mammalian receptors. The biological role of fish PTH remains unknown but will undoubtedly be of interest to evolutionary biologists.







**Figure 2** Schematic representation of the human PTH/PTHrP receptor and its gene organization. Amino acids are shown in single-letter code; the amino terminus of the receptor is at the top; bars indicate boundaries between each of 14 coding exons; exon 5 encodes the putative signal peptide. (Reproduced with permission from Elsevier (Jüppner *et al.* 2000)).

### PTH receptors and hormone action

An important breakthrough in understanding the physiological role of PTH and testing its molecular and cellular actions occurred when the receptor was successfully cloned in 1991 (Jüppner *et al.* 1991, Abou-Samra *et al.* 1992). Since that time, receptors for PTH from many additional species have been cloned (reviewed in Jüppner *et al.* 2000). Because of the diverse actions of PTH in multiple target tissues and due to *in vitro* evidence for multiple second messengers of hormone action from studies with cellular membrane fractions enriched in the PTH receptor (prior to its cloning), it was initially thought that several different receptors would be found to mediate some of these pleotropic actions of this peptide hormone. It was therefore somewhat surprising when the initial cloning approaches in several species led to detection of only a single G protein-coupled receptor, now referred to as the common PTH/PTHrP receptor, or PTHR1

(Fig. 2). This receptor mediates most of the traditional actions of PTH in mineral ion homeostasis and is critical to its actions on bone and kidney. Although it is beyond the scope of this review to discuss, there are other receptors for both PTH and PTHrP; however, there are a variety of biological actions ascribed to them, usually involving interaction with portions of either PTH or PTHrP beyond the amino terminal 34 residues (Jüppner *et al.* 2000). One of these additional receptors of particular interest is the carboxyl terminal PTH receptor that responds to C-terminal fragments of PTH (which are both generated by peripheral metabolism of the secreted intact hormone and by release from the parathyroid gland itself). As noted below, this ligand/receptor system may function as an antagonist of the actions of amino-terminal PTH and the PTHR1.

PTHR1, (shown in Fig. 2) belongs to a distinct family of G protein-coupled receptors. After the cloning of the original receptors from several animal species, cDNAs

**Figure 1** (A) Alignment of the amino acid sequences of known PTH (left panel) and PTH-related peptide (right panel) species. Conserved residues are shaded; numbers indicate the positions of amino acids in the mammalian peptide sequences. The figure does not include all recent PTH molecules such as zebrafish PTH. (B) Alignment of the (1–34) amino acid sequences of all known vertebrate PTH and PTHrP species, as well as fugu PTHrP and bovine TIP39. Amino acid residues that are conserved in all PTH and PTHrP species are shown in white boxes. Amino acid residues found in either fugu PTHrP or bovine TIP39 that are also found in all known PTH species are shown in black boxes with white letters, and residues found either in fugu PTHrP or in bovine TIP 39 that are also found in all PTHrP species are boxed; numbers indicate amino acid positions in mammalian PTH or PTHrP. Question marks refer to sequence positions in fugu fish PTHrP not definitely deduced from nucleotide sequence. (Reproduced with permission from Elsevier (Jüppner *et al.* 2000)).

encoding human PTHR1 as well as mouse, rat, chicken, pig, dog, frog and several PTH/PTHrP receptors from fish were isolated. The gene encoding the PTHR1 is located on chromosome 3 in humans. The gene involved in its synthesis has a total of 14 exons, the contributions of each of which are shown in Fig. 2. Family B receptors have a long amino terminal extracellular domain which is critical for binding peptide ligands such as PTH.

The cloning of the receptor and the intense studies of structure–activity relations with the PTH ligand and receptor (the latter by mutagenesis) have provided further tools with which to examine the cellular biology of PTH action (Bergwitz *et al.* 1996, Iida-Klein *et al.* 1997, Jüppner *et al.* 2000, Shimizu *et al.* 2000, 2001, 2002). These insights from cellular and molecular biology complement a variety of careful *in vivo* studies to help provide a more complete picture of the physiological role of the hormone *in vivo*. PTH acts in the kidney to increase the synthesis of  $1,25(\text{OH})_2\text{D}$  and thus indirectly increase intestinal calcium absorption. The hormone regulates renal calcium and phosphate transport, the latter action being important to support the overall homeostatic role of PTH (Jüppner *et al.* 2000). When calcium is needed (as with calcium-deficient diets or vitamin D insufficiency) calcium is mobilized from bone by increased PTH. The phosphate is not needed typically, since its dietary lack is infrequent, so PTH promotes phosphate excretion by blocking its reabsorption. PTH also works at distal tubular sites in the kidney to lower the amount of urinary calcium excreted; with calcium deficiency, less calcium is lost because PTH increases renal calcium reabsorption. PTH affects a wide variety of specialized bone cells, including osteoblasts and stromal cells. It also has important actions on osteoclasts, but those are indirect and are mediated through osteoblasts. A number of cell lines of osteoblasts and stromal cells and specialized tissue culture systems have evolved to study the interaction between the different cell types and their role in bone formation and bone resorption. Through its abundant receptors on osteoblasts, PTH has a variety of actions that are directly involved in promoting bone formation but physiologically, most importantly, to stimulate osteoclast differentiation and development and ultimately increased bone resorption. It is the latter action which has been traditionally associated with PTH – i.e. bone resorption. As noted below, however, the action to promote osteoblast activity directly has been exploited pharmacologically in the paradoxical but effective use of PTH in osteoporosis (see below).

### The heterogeneity of PTH: biological significance of PTH metabolism

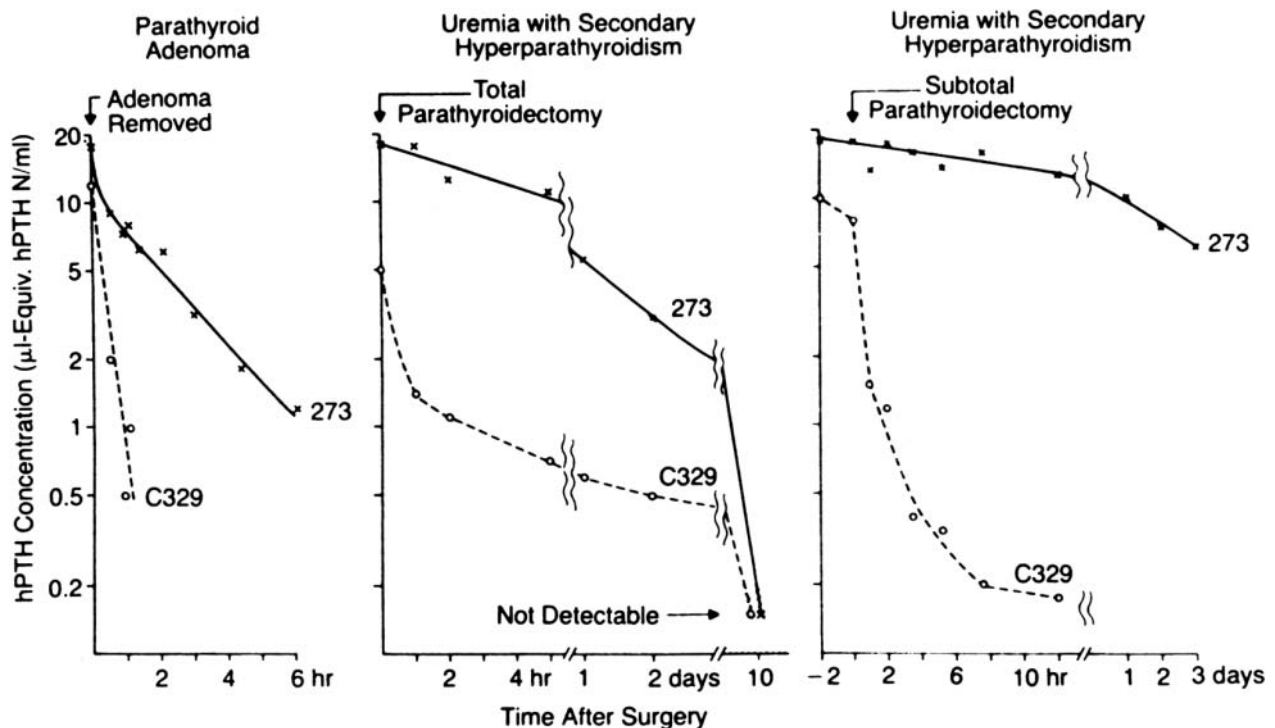
Figure 3 is of historical significance because it illustrates a vexing chapter in understanding the nature of circulating

PTH. Shown is the classic study of Berson and Yalow in which they applied several radioimmunoassays they had developed to analyze PTH in the circulatory system (Berson *et al.* 1963, Berson & Yalow 1968). Their development of radioimmunoassays for PTH represented a great advance in overall physiological studies of the hormone both in animals and in patients.

The availability of PTH radioimmunoassays eventually proved extremely helpful in the differential diagnosis of hypercalcemic or hypocalcemic disorders (Nussbaum *et al.* 1987, Nussbaum & Potts 1991, Jüppner *et al.* 2000, Jüppner & Potts 2002). The surprising findings of Berson and Yalow summarized in Fig. 3, however, were that different antibodies raised to the same PTH polypeptide could result in entirely different impressions of the rate at which the hormone concentration fell; for example, after removal of a parathyroid adenoma. By definition, this meant heterogeneity in circulating PTH. From the many investigations that followed, it is now apparent that in addition to the full-length polypeptide PTH(1–84), which is the biologically active hormone, much of the circulating hormone is fragmented, and these fragments lack biological activity (at least with regard to the PTHR1-mediated regulation of mineral ion homeostasis) (reviewed in Jüppner *et al.* 2000). C-terminal PTH fragments are both produced in and released from the parathyroid gland, but they are also derived from circulating intact hormone by efficient high capacity degradative systems in peripheral sites, especially the liver and kidney. Hence, the results shown in Fig. 3 could be understood, in retrospect, to be the result of different epitopes recognized by each particular antisera used in the studies, all raised against intact PTH (different recognition sites were recognized by each immunized animal) i.e. different epitopes. Depending on the exact size as well as the concentration of each PTH fragment, the apparent PTH (fragment) content would differ with different antisera.

Much work was expended by a number of laboratories in determining the nature of PTH metabolism, the origin of the circulating fragments (glandular versus peripheral), the chemical properties, and their overall biological significance (reviewed in Jüppner *et al.* 2000). It was concluded that the circulating fragments were all inactive and were merely an impediment to accurate measurement of the important biologically active form of the molecule. There appeared to be no significant concentration of circulating and biologically active amino terminal fragments; these latter appeared not to have survived the cleavage processes in the peripheral sites. Eventually, therefore, improved radioimmunoassays were developed that selectively measured only the intact PTH(1–84) molecule (believed then to be the only circulating molecular species of significance). Why this elaborate process of hormonal metabolism occurred seemed irrelevant. The newer immunologic techniques were derived from principles enunciated by Ekins (1981), who championed





**Figure 3** Disappearance of immunoreactive PTH from plasma after parathyroidectomy in patients with primary or secondary hyperparathyroidism. Plasma samples were assayed with antiserum C329 and antiserum 273, with an extract of a normal human parathyroid gland used as a standard (hPTH N) and  $^{125}\text{I}$ -bovine PTH used as a tracer. Plasma concentrations of hormone are given as microliter equivalents of the plasma standard of hPTH. (Reproduced with permission from the Endocrine Society, copyright 1968 (Berson and Yalow 1968)).

double antibody methods and who argued for intrinsic superiority of such approaches, even in the absence of heterogeneity of the measured compound, as in the case of PTH. Two different antibodies are employed, one to capture circulating hormone molecules and the other, which is radiolabeled, to detect hormone. With PTH, the intact molecule and all the carboxyl fragments are trapped by the capture antiserum, but the radiolabeled detection antibody was selected to have as its epitope the amino terminal portion of the molecule. This approach meant that only intact PTH(1–84), captured by the first and detected by the second, would be measured; fragments missing the amino terminal portion of PTH would not register. Such assays have greatly improved the sensitivity and specificity of PTH measurements (Nussbaum & Potts 1991). However, the work of D'Amour and colleagues (Brossard *et al.* 1996, Lepage *et al.* 1998, Nguyen-Yamamoto *et al.* 2001) and Slatopolsky *et al.* (2000) in the last decade has opened a new chapter in PTH metabolism.

The studies of D'Amour's group in using detection antibodies that react with the extreme amino terminus of PTH showed that there were PTH molecules nearly as large as PTH(1–84) but missing approximately the first six

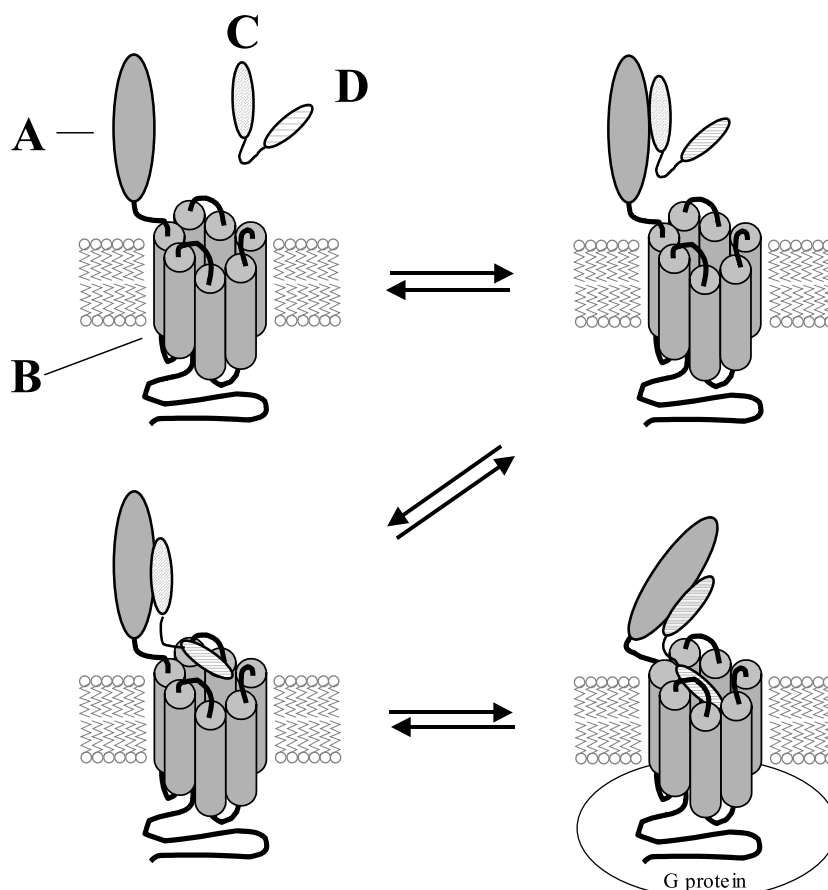
residues of the peptide – that is, they were fragments such as PTH(7–84). The concentration of these fragments relative to that of PTH(1–84) is higher in patients with renal failure and, to some extent, in those with primary hyperparathyroidism. Their relative concentration invariably rises in all patients studied when hypercalcemia is induced and PTH(1–84) concentrations are suppressed. It is known that there is active proteolysis of PTH within the gland, a phenomenon whose significance was never completely understood. Both D'Amour's and Slatopolsky's groups demonstrated that PTH(7–84) given *in vivo* to animals would block the hypercalcemic action of PTH(1–84). The significance of all these observations is far from clear; multiple laboratories are working on the problem. New assays have been developed which have as the detection antiserum epitopes that recognize the extreme amino terminus and therefore detect only intact PTH(1–84). It is not clear at this time whether the newer assays will be of greater discriminant value in certain disease conditions, such as renal failure in which adynamic bone disease occurs (believed to be due to excessive suppression of PTH by therapies such as supplemental calcium and vitamin D) (Jüppner & Potts 1991). The recent studies do, however, lead to the speculation that in

the parathyroid gland, when hormone secretion should be and is suppressed, the gland then secretes an inhibitor of PTH action. Much work would be needed to establish the validity of such a speculation, but this particular chapter in PTH history is just being written; whether it will prove to be an important new feature of understanding PTH action remains unsettled.

### Structure/activity relations in PTH and its receptor

The work on defining the essential features of hormone/receptor interaction (here referring to PTHR1, the principal receptor for the traditionally recognized actions of the hormone on calcium and bone) has become an area of intense interest both in basic studies by academic groups and, in largely unpublished studies, by several pharmaceutical firms looking for a peptidomimetic. Scores of synthetic peptide fragments and analogs have been used to determine the essential pharmacophore for the PTH ligand and/or the capacity of some PTH analogs to signal selectively (Takasu *et al.* 1999, Shimizu *et al.* 2000, 2001, 2002). The PTH receptor clearly has multiple signaling pathways, including the most prominent one,  $G_{s\alpha}$ -dependent, cAMP generation via adenylyl cyclase, as well as inositol triphosphate generation, a non phospholipase C-dependent protein kinase C action, and calcium transients that at least in certain cell types are not dependent on cAMP generation (Jüppner *et al.* 2000). In the process of these studies, mutagenized forms of the receptor have also been used to map regions of complementarity between ligand and receptor. The current state of this work was well reviewed recently (Gardella & Jüppner 2001). Gardella and colleagues have established a number of critical features of hormone/receptor interaction, which may apply to the entire class B family of peptide hormone G protein-coupled receptors. This work is of fundamental interest in understanding hormone/receptor interaction but also has great practical significance, since the possibility of developing a small molecule equivalent, a peptidomimetic for PTH, might emerge from such careful studies of the essential features of the critical step(s) in forming the active bimolecular complex of hormone and receptor. A working hypothesis has emerged concerning the interaction of different regions of the biologically active amino terminal 34-aminoacid region of the peptide with the receptor. The large extracellular domain of the receptor is referred to as the N-domain. The remainder, referred to as the J-domain, includes the portion of the receptor that contains the three extracellular loops that connect the seven transmembrane-spanning helices, the helices themselves, and three intracellular domains, with a large terminal intracellular domain (Fig. 2) (Bergwitz *et al.* 1996, Hoare *et al.* 2001). The J-domain is the functional

portion of the receptor. There is evidence that the PTH peptide can interact with these two regions somewhat independently. The carboxyl-terminal portion of the parathyroid ligand binds to the N-domain of the receptor and provides critical docking interactions with the receptor that makes it possible for the otherwise weakly binding amino terminal domain of the ligand to associate with the J-domain of the receptor (Fig. 4). The amino terminal domain of PTH, particularly the first several residues, has long been known to be critical for activation. The model, supported by a large amount of experimental data, indicates that this amino terminal portion of the PTH interacts with critical residues within the J-domain of the receptor, thereby inducing or selecting the active conformation that binds preferentially G proteins and thus begins the cascade of second messenger-mediated hormone-specific events within target cells. An important development has been the generation of a highly modified and potent version of the (1–14) amino terminal portion of PTH (Fig. 1). The (1–14) region is only weakly active if tested by itself because it lacks effective binding to the receptor normally provided by the multi-site interaction involving the carboxyl-terminal portion of the (1–34) ligand with the extracellular domain of the receptor. Design of the more potent molecule took advantage of evidence that the amino terminus of the full-length PTH(1–34) ligand, which does not initially have much secondary structure (i.e. alpha helix), develops a highly ordered secondary structure upon contact with the receptor (Gardella 2005). Among the amino acid changes tested, therefore, were paired substitutions of a conformationally constrained amino acid,  $\alpha$ -amino-isobutyric acid (AIB) at positions one and three. These changes together provided a 100-fold increase in the potency of the otherwise weakly active PTH(1–14). Other activity-enhancing substitutions had been determined by systematic replacement, particularly in regions 10–14, where glutamine, homoarginine, alanine and tryptophan were substituted for the native sequence at positions 10, 11, 12 and 14 respectively (Fig. 1). The resulting highly modified peptide, Aib1,3, Gly10, Har11, Ala12, Tryp14, PTH(1–14) is 100 000-fold more potent than unmodified (1–14) and in many cell-based assays with high receptor number is as potent as native PTH (Gardella & Jüppner 2001, Shimizu *et al.* 2001). In fact, it is equivalently active with the wild-type receptor or constructs generated in which only the J-domain is present. The substitutions conferred activity to even shorter peptides previously found to be inactive, such as PTH(1–11) (Shimizu *et al.* 2001). These and other studies (again beyond the scope of this review) seem to establish firmly a general mechanism of hormone–receptor interaction. The results are also clearly consistent with a strong evolutionary conservation of this portion of the PTH. Much evidence in the PTHR1 field, as well as in other fields of study of G protein receptors and their agonists, increasingly convey the notion that the activation process



**Figure 4** Model for modulation of ligand binding to the PTH1 receptor by G protein. The C-terminal portion of the ligand (C) interacts with the N-domain of the receptor (A). Subsequently, the N-terminal portion of the ligand (D) binds to the J-domain of the receptor (B). Receptor/G protein interaction (lower right) increases the affinity of the ligand/J-domain interaction (modified to a more closed receptor conformation). Reciprocally, interaction of the ligand with the J-domain increases the affinity of receptor for G protein, stimulating G protein activation. Binding of G protein to the other states of the receptor (R and RLN) has been omitted for clarity. (Reproduced with permission from the American Society for Biochemistry and Molecular Biology (Hoare *et al.* 2001)).

is likely to be a multi-step concerted process, so that different activated states of the PTHR1 could be responsible for a distinct set of signal transduction pathways and/or postactivation responses.

### PTH and the therapy of osteoporosis

The most recent chapter in the history of PTH is its use as the most effective current therapy for osteoporosis. This fact is quite surprising and clearly paradoxical (See Table 2). Hyperparathyroidism is invariably associated with bone loss, not bone gain (even though the severe bone disorder, osteitis fibrosis cystica – or von Recklinghausen's disease – is itself rare). How administration of PTH, which causes bone loss in hyperparathyroidism can

be a cure for osteoporosis is the paradox. Strictly, of course, PTH therapy does not cure osteoporosis; rather, it simply greatly restores bone mass, especially trabecular bone, increases bone strength and dramatically (and quickly) reduces fracture incidence (Reeve *et al.* 1980, Tam *et al.* 1982, Lindsay *et al.* 1997, Lane *et al.* 1998, Dempster *et al.* 2001, Neer *et al.* 2001).

In general, the therapy of osteoporosis has been a dramatic success story, particularly in the last several decades. There are multiple effective therapeutic agents (Rodan & Martin 2000). Prior to the latter part of the 20th century, osteoporosis was largely untreatable. The disease usually declared itself by one or more spontaneous or pathological fractures, often in the spine or in the hip, the latter being especially catastrophic with regard to medical consequences (Delmas & Chapurlat 2005).

**Table 2** Paradox of PTH biological action: therapeutic use vs physiological function

Homeostatic role	Sustained elevation of PTH can maintain blood calcium against challenge of prolonged calcium deficiency by withdrawal from bone ‘bank’, reduces bone mass.
Therapeutic role	Deliberate, short pulses of PTH dramatically build bone mass. a. Continuous elevation in PTH blood levels (>2 hrs): ↓ bone mass b. Intermittent elevation in PTH blood levels (<2 hrs/day): ↑ bone mass

Multiple factors contributed to the improved diagnosis and therapy of this disease, including the development of reliable, non-invasive methods of measuring bone mass (Njeh *et al.* 2005). The second great advance has been the development of effective antiresorptive therapies, principally bisphosphonates (Delmas & Chapurlat 2005, Rodan & Martin 2000).

PTH represents, however, much more than simply an additional agent for the therapy of osteoporosis. The mechanism of action of PTH establishes it as the first in a class of anabolic agents for osteoporosis. An anabolic agent is one that directly stimulates bone formation and is therefore to be distinguished from antiresorptive agents which act by blocking bone resorption, allowing the endogenous rate of bone formation to build bone since it is now unopposed by resorption. The difference between the lowered bone resorption rate and the continuing intrinsic bone formation results in an eventual gain in bone mass, an effect that occurs more slowly than that seen with PTH.

The first evidence, not at all appreciated at the time, that PTH increases bone mass occurred many decades ago at the time that the pathophysiological significance of excess PTH (hyperparathyroidism) was just being worked out, as outlined above. The first paper to report an

increase in bone mass achievable by daily injections of PTH was published during studies of the use of the then newly available PTH in the therapy of lead intoxication (Bauer *et al.* 1929) (See Table 3). Because excess PTH in humans due to parathyroid tumors was known to cause severe bone loss, the results in rats appeared to be an anomaly not pertinent to human medicine. The observation made by Bauer *et al.* was, however, confirmed by Selye several years after (Selye 1932). After a lag of 40 years, interest in the topic resurfaced. The late pharmacologist, John Parsons, was one of the major proponents of the potential of PTH for the therapy of osteoporosis, arguing that the paradox was merely the difference between results with continuous elevation of PTH (i.e. bone loss) and intermittent, short elevations of PTH (i.e. bone mass increase). Intermittent high PTH is anabolic for bone, while continuous elevation is catabolic (Rodan & Martin 2000, Harada & Rodan 2003). The only receptor for PTH is on the osteoblast; its activation by hormone may prolong osteoblast life and increase its activity, leading to bone formation. The signaling by several intermediate messengers from osteoblast to osteoclast to stimulate the latter and resorb bone occurs less rapidly than the initial direct stimulation of the osteoblast. Hence, elevations that are transient may stimulate the

**Table 3** History of PTH as an anabolic agent

Date	
1929	MGH: Bauer, Aub, Albright (Bauer <i>et al.</i> 1929) PTH ↑ bone mass in rats
1932	Confirmed by Selye (1932) After these reports, no further studies for 40 yrs
1965–1972	NIH & MGH: isolation, structure, synthesis of PTH provides pure material for clinical study
1975	Rapid improvement in techniques for accurate assessment of bone mass Resumption of clinical interest US, England, France: trials with PTH in osteoporotic patients begin
2001	Striking efficacy found in bone mass and fracture prevention in controlled international study*
2002	Approved by US FDA for therapy of osteoporosis

\* Commercial sponsorship finally developed despite lack of patent protection.  
Eli Lilly initiates placebo-controlled fracture prevention trial in 1997.  
Osteosarcoma develops in rats in Lilly toxicology study; trial halted in 1998.  
MGH, Massachusetts General Hospital; NIH, National Institutes of Health; FDA, Food and Drug Administration.



osteoblast anabolic activity while not yet triggering the coupled catabolic response through osteoclasts. Timing is critical in the administration of PTH. An important study by Dobnig and Turner (1997) in test animals with controlled rates of administration of hormone showed that less than two hours of exposure to PTH elicited the anabolic response and longer than two hours the catabolic response. What remains unsettled is the exact cellular mechanisms by which the favorable anabolic response occurs with short exposure to increased PTH levels.

The definitive clinical trial in humans involved over 1600 postmenopausal women with prior vertebral fractures. PTH strikingly reduced fracture incidence compared with the placebo group, overall reducing fractures by 70% (Neer *et al.* 2001). New nonvertebral fractures, fragility fractures, were also reduced by approximately 50%. This therapeutic efficacy led the US Food and Drug Administration to approve PTH for use in osteoporosis even though in extended toxicology studies by the sponsor (lifelong exposure to PTH in a cancer-prone rat strain) osteosarcomas developed near the end of the lifetime of the animals. Since the therapeutic use of PTH is limited to two years, and with other reassuring evidence, the osteosarcomas in rats were deemed not relevant for PTH use in humans (Neer *et al.* 2001). This report was the culmination of several decades of investigator-initiated trials, the first major one was reported by Reeve *et al.* in 1980, which had shown striking benefits in bone mass increase. These trials were of insufficient size, however, to estimate fracture incidence (Reeve *et al.* 1980).

The beneficial effects of PTH are primarily in trabecular bone, but this improvement in trabecular bone translates to improved bone strength, particularly in the vertebrae but also in the hip. There does not seem to be much improvement in cortical bone – for example, at the wrist – but in vertebral bodies cortical bone is increased, as well as trabecular bone itself (Dempster *et al.* 2001).

The success with PTH, as is usual in biomedical science and medicine, raises a new set of interesting questions, the answers to which should further advance the field. Why is cortical bone, at least in some sites, less responsive to hormone? Must bone formation and bone resorption be coupled, or can one have an anabolic response without prior increased bone resorption? The data on PTH is equivocal in that bone resorption markers do rise although not as rapidly as bone formation markers as the weeks of therapy with the hormone continue. Can orally active agents replace PTH and perhaps even surpass its efficacy? The need for continuous daily subcutaneous injection has limited the use of the hormone, largely to patients with already established and severe osteoporosis. Can the effectiveness of PTH be augmented by combining an anti-resorptive with intermittent PTH? This question remains open, but one approach has proven to be ineffective; when bisphosphonates (alendronate) and PTH are given simultaneously, the bisphosphonate retards the effect of

PTH significantly (Khosla 2003). Other combinations of an anti-resorptive and PTH might be additive or synergistic; however, it may be possible that bone formation and bone resorption must always be coupled to a certain degree, so that bone formation will always be impaired, to some extent, if bone resorption is blocked. What are the critical steps in the action of PTH beyond receptor activation that result in specific cellular responses that cause an anabolic skeletal effect? Can these critical downstream effector steps in PTH action be identified and serve as targets for new therapeutic agents (Rodan & Martin 2000)?

Major developments have occurred in bone biology in recent years. Research has defined the cellular lineages involved in bone formation and bone resorption (the osteoblasts and marrow stromal cells, the osteocytes, and osteoclasts with their precursor cell types), and the method of intercommunication between bone cells. Many new potential targets for stimulating bone formation are being recognized. The hope is that eventually all of the current agents, including the antiresorptives and even PTH, will be surpassed in convenience and effectiveness by other compounds, preferably orally acting, that are superior types of anabolic agents for bone. Such developments seem predictable, based on new advances in bone research and the intense interest of biotechnology and pharmaceutical firms in this field's potential.

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