Chapter 13. Regulation of Calcium, Magnesium, and Phosphate Metabolism

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INTRODUCTION

The mineral ion homeostatic system maintains Ca, Mg, and P (because P does not exist as free P in biological systems, it will be referred to as phosphate and abbreviated PO_4) distribution and levels in the extracellular fluid and intracellular compartments while simultaneously providing sufficient absorption and retention to meet skeletal mineral requirements. Homeostasis is achieved by the coordinated interaction of the intestine, the site of net absorption; the kidney, the site of net excretion; and the skeleton, the largest repository of these ions in the body. Mineral fluxes across intestine, bone, and kidney and in and out of blood are regulated by PTH and 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], the hormonal or active form of vitamin D (also called calcitriol). There is emerging evidence that fibroblast growth factor (FGF)-23 may also be an important physiologic regulator of PO_4 homeostasis. External balance may vary with the stages of the life cycle, skeletal mineral requirements, supply of the minerals in the diet, and by a variety of disorders. For example, balance is neutral in adult, nonpregnant humans who have no daily net gain or loss of body Ca, Mg, or PO_4. Mineral balance becomes positive (intake and retention exceed urinary and intestinal losses) during skeletal growth during childhood and adolescence and during pregnancy and lactation. Negative balance (gastrointestinal and/or urinary losses exceed intake and retention) may occur during high rates of bone remodeling during estrogen deficiency, with aging, and with diseases such as hyperthyroidism and primary hyperparathyroidism. The adjustment or adaptation of the homeostatic system to maintain balance is mediated by PTH and $1,25(OH)_2D_3$ through changes in intestinal and renal tubule mineral transport. In the following sections, the distributions and fluxes of Ca, Mg, and PO_4 and hormonal regulations of mineral ion transport and balance are discussed.

CALCIUM

Total Body Distribution

Total body Ca content in adults (Fig. 1) is about 1000 g, of which 99% exists as the hydroxyapatite [$Ca_{10}(PO4)_6(OH)_2$] crystal in the mineral phase of bone. The crystal contributes to the mechanical weight-bearing properties of bone and also serves as a Ca and PO_4 reservoir that can be rapidly mobilized to support the numerous biological systems in which Ca or PO_4 are cofactors and regulators. The remaining 1% of total body Ca is in soft tissue and the extracellular fluid (ECF) space including blood. Serum total Ca concentration of 10^{-3} M exists in the ionized (50%) and protein-bound (40%) states and complexed mainly to citrate and PO_4 ions (10%). The biological actions of Ca are attributed to the ionized fraction, which is readily exchangeable with pools of Ca in bone, blood, and intracellular sites.

Cellular Distribution

Ionic cytosol Ca is maintained at about 10^{-6} M. Because blood and ECF Ca is 10^{-3} M, the 1000-fold chemical gradient favors Ca entry into the cell. The differential electrical charges across the cell plasma membrane of 50-mV gradient (cell interior negative) creates an electrical gradient that also favors Ca entry. Therefore, the major threat to cell viability is excessive Ca influx from the extracellular space along the electrochemical gradients. The defense against excess Ca influx into cells includes extrusion of Ca from the cell through energy-dependent Ca channels, Ca-dependent ATP-driven Ca pumps, and Na-Ca exchangers and active uptake into organelles including the endoplasmic reticulum and mitochondria. Ca-binding proteins and Ca transport proteins facilitate Ca transport into these cellular organelles. They also serve to buffer excess calcium preventing cell death. Ca-rich mitochondria and endoplasmic reticulum also serve as reservoirs to maintain cytosolic Ca when levels fall. Ca bound to the plasma membrane and the organelles may also be released in pulses in response to activation of receptors on the external surface of the plasma membrane.

Cell Ca homeostasis varies by cell function. For example, Ca facilitates the linking of excitation and contraction in skeletal and cardiac muscle through mobilization of the large Ca intracellular stores of the sarcoplasmic reticulum. In nonskeletal cells, Ca serves as a signal transducer, mediating signaling from activated plasma membrane receptors to carry out a variety of functions such as hormone secretion, neurotransmission, and kinase phosphorylation.

Homeostasis

Because diet Ca intake and skeletal Ca requirements vary widely from day to day and across the various stages of the life cycle, the homeostatic system is constantly adjusting to deliver sufficient Ca, Mg, and PO_4 from intestine and kidney into the ECF and blood and then to bone to meet changing skeletal growth requirements without disturbing the serum ionized Ca concentration [Ca^{2+}]. The serum Ca^{2+} fraction controls cellular biological functions, and therefore the homeostatic system maintains serum Ca^{2+} at the expense of BMC.

Serum Ca^{2+} may increase from Ca influx from intestinal absorption or bone resorption and decrease with Ca efflux into bone mineralization sites, secretion into the intestinal lumen, or filtration at the renal glomerulus and secretion along selected segments of the nephron. A decline in serum Ca^{2+} is potentially more likely and is therefore defended against by all of the actions of PTH. PTH secretion is regulated by the parathyroid cell plasma membrane Ca-sensing receptor (CaSR), which detects ambient serum Ca^{2+} and so regulates minute-to-minute PTH secretion. PTH increases Ca influx into the extracellular space through enhanced renal tubule reabsorption of filtered Ca that occurs within minutes; increased osteoclastic- and osteocytic-mediated bone resorption that appears within minutes to hours; and stimulation of intestinal Ca absorption indirectly through increased renal proximal tubule $1,25(OH)_2D_3$ synthesis that appears by 24 h after PTH secretion. Hypercalcemia suppresses CaSR signaling and thereby suppresses PTH secretion. Elevated serum Ca^{2+} stimulates distal nephron CaSR, which reduces net tubule Ca reabsorption, increases urine Ca excretion, and thus lowers serum Ca^{2+} to normal.

The authors have reported no conflicts of interest.

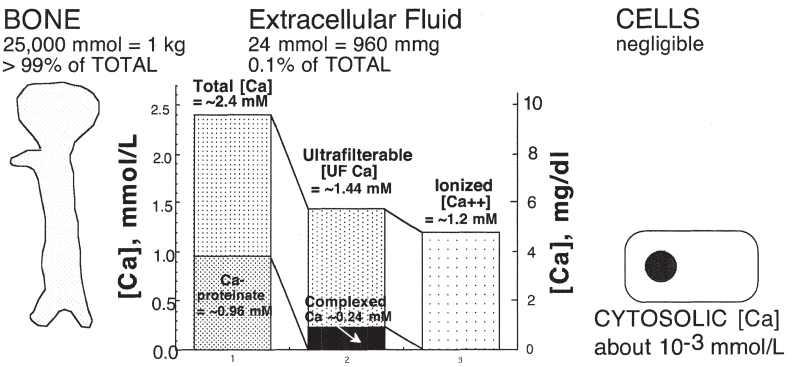

FIG. 1. Ca content and distribution in a 70-kg adult.

Intestinal Absorption

Ca intake is largely dependent on dairy product intake. Ca intake varies from a very low of 5.0 mmol (200 mg) per day to well above the recommended intake of 25 mmol (1000 mg) per day for adult males <65 years of age and premenopausal women. Net Ca absorption measured by the external balance technique shows the relationship between net absorption and dietary Ca intake (Fig. 2). Negative Ca balance appears when Ca net absorption falls below 5.0 mmol (200 mg) per day. Ca requirements increase with age, presumably to compensate for the decline in intestinal Ca absorption with age in both men and women. High rates of absorption occur in children, adolescents, young adults, and during pregnancy and lactation. When the diet is low in Ca, the efficiency of the absorptive process is enhanced to absorb the greatest portion of ingested Ca. Efficiency of absorption declines with age or when Ca intake is high. In humans, the efficiency of absorption can be measured as fractional Ca absorption using external 6-day balance studies, segmental perfusion after intubation with a multiple lumen tube, and single- and double-Ca stable- or radio- isotope kinetics. These studies reveal that only a fraction of dietary Ca is absorbed, ranging from 20% to 60% (Fig. 3). The range reflects age, Ca intake, skeletal requirements, vitamin D status, the state of the homeostatic system, and the bioavailability of Ca contained in foods. Despite the array of Ca absorption techniques used in clinical investigations, none are available for routine patient management. Fecal Ca losses estimated from balance studies are between 2.5 and 5.0 mmol (100 and 200 mg) per day (Fig. 3). The origins of fecal Ca include unabsorbed dietary Ca and secreted Ca contained in pancreatic and biliary juices and mucosal secretion. Fecal Ca is not regulated by hormones or serum Ca.

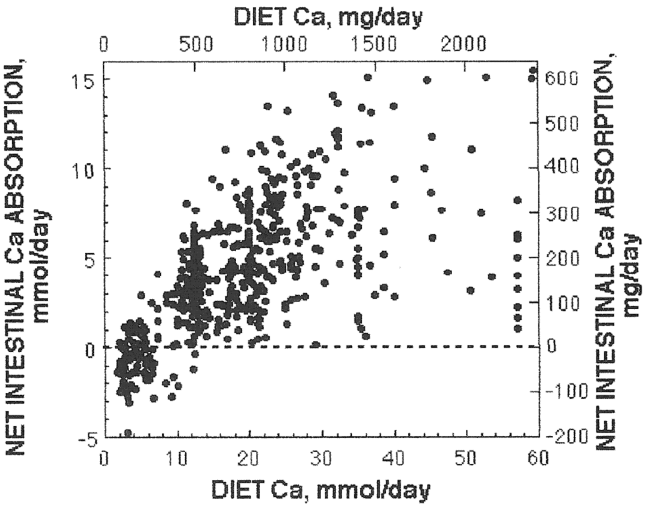

FIG. 2. Net intestinal Ca absorption in humans as measured by the external balance method in relation to dietary Ca intake. Adapted with permission from top right panel of Fig. 30.6. In: Coe FL, Favus MJ (eds.) Disorders of Bone and Mineral Metabolism, 2nd ed. Lippincott Williams & Wilkins, Philadelphia, PA, USA, 2002, pp.678.

Sites, Mechanisms, and Regulation of Absorption

Transit time is rapid in the duodenum and jejunum; however, the large surface area in these regions are responsible for ~90% of Ca absorbed during adequate Ca intake. Increased Ca requirements stimulate Ca active transport primarily in duodenum and ileum and to a lesser extent in jejunum and all regions of colon to increase fractional Ca absorption from 25–45% to 55–70%.

The relationship between net intestinal Ca absorption (diet Ca intake − fecal Ca excretion) and diet Ca intake is derived from metabolic balance studies (Fig. 3). To maintain neutral or positive Ca balance, healthy adults require about 10 mmol (400 mg) Ca intake per day, because net Ca absorption is negative (fecal Ca exceeds diet Ca intake) or less than zero when diet Ca is less than 5.0 mmol (200 mg) per day (Fig. 2). As diet Ca intake increases from very low levels (<5.0 mmol or 200 mg/day), net Ca absorption increases and begins to plateau as Ca intake approaches 25 mmol (1000 mg) per day (will provide net absorption of about 7.5 mmol or 300 mg/day). The curvilinear relationship between net Ca absorption and Ca intake reflects the sum of two absorptive mechanisms: a cell-mediated, saturable active transport; and a passive, diffusional, paracellular absorption that is driven by transepithelial electrochemical gradients. The wide variation in net Ca absorption among healthy adults for any given level of Ca intake (Fig. 2), especially when Ca intake is above the 15–20 mmol (600–800 mg) per day range, is most likely caused by variation in the activity of the active transport component. Intestinal perfusion studies in humans show that net Ca absorption increases with luminal Ca concentration with a tendency to plateau at higher concentrations. Adults fed a low Ca diet for 1 month have greater net Ca absorption at the same level of luminal Ca concentration as those fed a diet adequate in Ca, which reflects stimulation of Ca active transport by $1,25(OH)_2D_3$. Very high Ca intakes increase primarily passive absorption, because serum $1,25(OH)_2D_3$ levels are low. Diffusional Ca flow along the paracellular pathway is in part dictated by the permeability of each intestinal segment, which is similar across duodenum, jejunum, and ileum, lowest in cecum, and intermediate across the colon. Major causes of increased and decreased Ca absorption are listed in Table 1.

At the cellular level, transcellular Ca transport (Fig. 4) is stimulated by $1,25(OH)_2D_3$ and upregulation of the intestinal epithelial vitamin D receptor (VDR). VDR-mediated increased expression of a number of vitamin D–dependent genes produce proteins that participate in the active transport process. Ca influx across the brush border membrane is facilitated by the

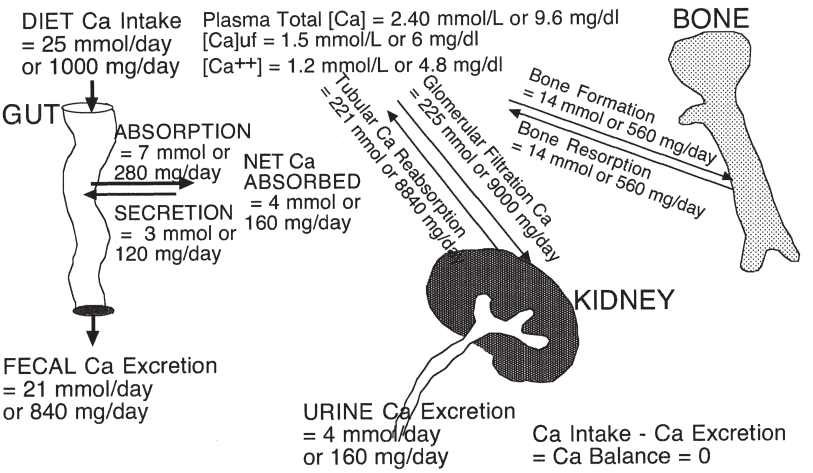

FIG. 3. Daily Ca turnover in a 70-kg adult.

channel created by the Ca transport protein 1 (TRPV6), which is induced by both $1,25(OH)_2D_3$ and estradiol independently through their cognate receptors. Within the cell (Fig. 4), Ca destined for transport is sequestered in vesicles containing the Ca binding protein calbindin 9 kDa. The vesicles then move to and fuse with the basolateral plasma membrane to begin the process of Ca extrusion out of the cell. Ca exit from the cell may involve both the low affinity, high capacity Na-Ca exchanger and an energy-dependent, high-affinity, limited capacity Ca, Mg-dependent ATPase (Fig. 4).

Renal Handling and Urinary Excretion

Filtration. Complexed and ionized Ca together are termed the ultrafilterable Ca and are freely filtered by the glomerulus with a Ca concentration of ~1.5 mM. The quantity of Ca filtered each day of over 270 mmol (10 g) is far greater than the Ca content of the entire ECF compartment and far more than net intestinal Ca absorption, which is ~4.0 mmol (160 mg) per day. To maintain neutral Ca balance, ~98% of the filtered Ca must be reabsorbed along the renal tubule. The substantial filtration followed by selective reabsorption allows precise control of excretion.

TABLE 1. CAUSES OF INCREASED AND DECREASED INTESTINAL Ca ABSORPTION

Increased Ca absorption	*Decreased Ca absorption*
Increased renal $1,25(OH)_2D_3$ production	Decreased $1,25(OH)_2D_3$ production
Growth	Hypoparathyroidism
Pregnancy	Vitamin D deficiency
Lactation	Vitamin D–dependent rickets, type I
Primary hyperparathyroidism	Chronic renal insufficiency
Idiopathic hypercalciuria	Aging
Increased extrarenal $1,25(OH)_2D_3$	Normal $1,25(OH)_2D_3$ production
Sarcoidosis and other granulomatous disorders	Glucocorticoid excess
B-cell lymphoma	Thyroid hormone excess
	Intestinal malabsorption syndromes
With normal $1,25(OH)_2D_3$ production	Increased renal $1,25(OH)_2D_3$ production
Idiopathic hypercalciuria	Low dietary Ca intake

Reabsorption. Approximately 70% of filtered Ca is reabsorbed in the proximal tubule through predominately passive mechanisms. About 20% of filtered Ca is reabsorbed in the loop of Henle. Little Ca is reabsorbed in the thin descending and thin ascending limbs of the loop. However, the thick ascending limb of the loop of Henle (TALH) is the sight of paracellular Ca reabsorption driven by the Na-K-2 Cl transporter. Loop diuretics such as furosemide impair Ca reabsorption in this segment by decreasing lumen-positive voltage created by the transporter. The basolateral membrane of these cells contains the CaSR. An increase in peritubular Ca stimulates the CaSR, which reduces lumen positive voltage and thereby reduces Ca reabsorption. Also along this segment is the tight junction protein paracellin 1. Mutations of paracellin 1 result in a selective defect in paracellular Ca and Mg reabsorption. The distal convoluted tubule reabsorbs ~8% of filtered Ca and is the major site of physiologic regulation of urine Ca excretion. Active Ca reabsorption against electrochemical gradients involves entry across the apical membrane through the highly Ca-selective renal epithelial Ca channel 1 (TRPV5). The

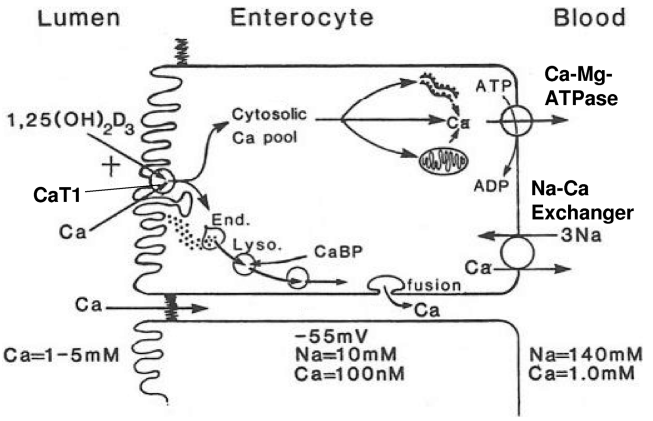

FIG. 4. Schematic representation of cellular and paracellular pathways for Ca transport across intestinal epithelium. Lumen-to-blood electrochemical gradients drive the paracellular pathway for Ca transport, and lumen-to-intracellular gradients are overcome by energy-dependent transcellular transport. The transcellular Ca sequestration model for intracellular Ca transport is shown. Molecules in bold are either ion channels, or transporters. CaBP is calbindin 9 kDa located within the Ca-transporting lysosomes. Reprinted with permission from Favus MJ 2002 Intestinal absorption of calcium, magnesium, and phosphorus. In: Coe FL, Favus MJ (eds.) Disorders of Bone and Mineral Metabolism, 2nd ed. Lippincott Williams Wilkins, Philadelphia, PA, USA, pp. 48–73.

channel is selectively more permeable to Ca than Na and is induced by $1,25(OH)_2D_3$, estradiol, and low Ca diet. Cytosolic Ca diffusion is facilitated by Ca binding to calbindin 28 kDa and calbindin 9 kDa, and active extrusion across the distal nephron plasma membrane is accomplished by the Na-Ca exchanger and a Ca-ATPase (PMCA1b). While Ca generally follows Na in this segment, reabsorption and excretion of Ca and Na can be dissociated. The collecting duct absorbs <5% of the filtered load. As a result of the reabsorption of Ca along the nephron, the final urine contains only ~2% of the filtered load.

Factors Affecting Reabsorption. Factors that may increase or decrease Ca excretion and their sites of action are listed in Table 2. Volume expansion increases and volume contraction decreases urine Ca and Na excretions through decreased and increased proximal tubule ion reabsorption, respectively. Increased dietary Ca increases urine Ca excretion with 6–8% of the increase in diet Ca appearing in the urine. Hypercalcemia increases ultrafilterable Ca but decreases glomerular filtration rate (GFR). In addition, hypercalcemia decreases proximal tubule, TALH, and distal convoluted tubule Ca reabsorption resulting in greater urinary excretion of Ca than Na. Ca activation of CaR in the TALH decreases Ca reabsorption, and Ca may also alter paracellin 1–regulated Ca and Mg permeability with decreased reabsorption of both ions. In the inner medulla collecting duct, the CaSR inhibits the water channel aquaphorin 2, which leads to polyuria, often observed in patients with hypercalcemia. PTH is the principle regulator of Ca reabsorption, with high PTH levels stimulating Ca reabsorption (Fig. 5). PTH reduces GFR and thus the filtered load of Ca, increases TALH active Ca transport, and opens the epithelial calcium channel (ECaC) in the distal convoluted tubule. Although PTH increases net Ca reabsorption, patients with primary hyperparathyroidism are often hypercalciuric, because increased tubule Ca reabsorption leads to hypercalcemia and an increased filtered load of Ca and resulting in hypercalciuria (Fig. 5). PTH-related peptide (PTHrP), which is secreted by a number of malignant cells, mimics the actions of PTH along the nephron. $1,25(OH)_2D_3$ actions on the kidney are complex and poorly understood. Vitamin D deficiency reduces Ca reabsorption independent of PTH levels. $1,25(OH)_2D_3$ increases CaSR expression, which decreases Ca reabsorption. Calbindin 28 kDa levels are increased and may increase Ca reabsorption. PO_4 administration reduces urine Ca excretion through increased distal Ca reabsorption and stimulation of PTH. Dietary PO_4 deprivation causes hypercalciuria in part by actions in the distal nephron. Acute and chronic metabolic acidosis increases urine Ca excretion, and alkalosis decreases urine Ca excretion. Endogenous acid production from the metabolism of sulfur-containing amino acids (methionine, cysteine) found in animal protein contributes to the postprandial increase in urine Ca.

Diuretics have variable effects on urine Ca excretion. Thiazide and related diuretics (hydrochlorothiazide, chlorthalidone), indapamide, and amiloride increase urine Na excretion and decrease urine Ca through an increase in renal tubule Ca reabsorption mainly in the distal convoluted tubule. These agents are useful in the treatment of Ca nephrolithiasis because they reduce hypercalciuria and urine Ca oxalate supersaturation. Loop diuretics (furosemide, torosemide, ethacrynic acid) increase urine Ca and Na excretion by inhibiting the Na-K-2 Cl transporter in the TALH. Hypercalciuria continues as long as urinary Na losses are replaced. These agents are useful in treatment of hypercalcemia.

Genetic disorders may increase or decrease renal Ca transport, including Bartter's syndrome associated with several mutations affecting TALH and hypocalciuria; Dent's disease caused by a defect in the proximal tubule affecting the chloride channel resulting in decreased tubule Ca reabsorption and hypercalciuria; inactivating mutations of the CaSR in familial hypocalciuric hypercalcemia (FHH) leading to increased renal tubule Ca reabsorption and hypocalciuria; activating mutations of the CaSR in autosomal dominant hypocalcemia associated with low PTH, decreased renal tubule Ca reabsorption, and hypercalciuria; and Gitelman's syndrome accompanied by hypocalciuria.

TABLE 2. FACTORS INFLUENCING RENAL Ca EXCRETION

Glomerular filtration
Increased
Hypercalcemia
Decreased
Hypocalcemia
Renal insufficiency
Tubular reabsorption
Increased
ECF volume depletion
Hypocalcemia
Thiazide diuretics
Phosphate administration
Metabolic alkalosis
Parathyroid hormone
Parathyroid hormone related peptide
Familial hypocalciuric hypercalcemia
Decreased
ECF volume expansion
Hypercalcemia
Dietary PO_4 deprivation
Metabolic acidosis
Loop diuretics
Cyclosporin A
Autosomal dominant hypocalcemia
Dent's disease
Bartter's syndrome

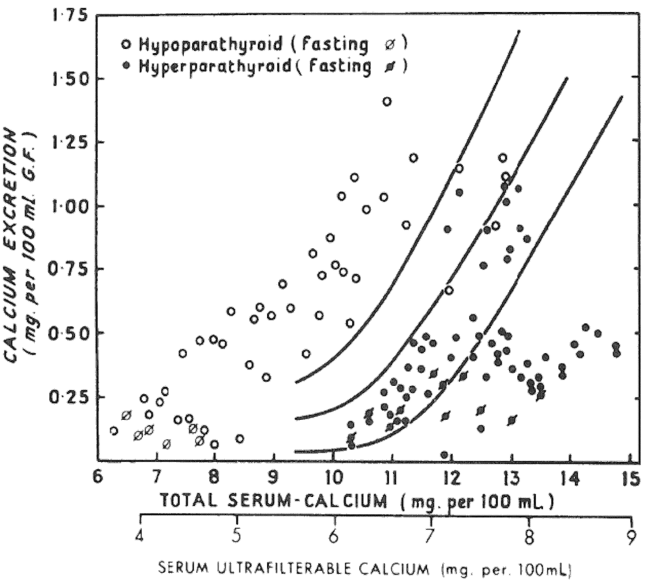

FIG. 5. Urinary excretion of Ca as a function of serum Ca concentration in normal subjects (solid line) and in patients with hypoparathyroidism (triangles) and hyperparathyroidism (circles). Dashed lines ± SD define normal range. Reprinted from Nordin BEC, Peacock M 1969 Role of kidney in regulation of plasma calcium. Lancet **2:**1280–1283 with permission from Elsevier.

MAGNESIUM

Total Body Distribution

Adult tissues contain about 1.04 mol (25 g) of Mg, of which 66% is located within the skeleton, 33% is intracellular, and 1% is within the extracellular compartment. Although Mg is a major constituent of bone, it is not a consistent component of the hydroxyapatite crystal structure. Mg is primarily on the crystal surface, and a portion is in equilibrium with ECF Mg. Mg is the most abundant divalent cation in the intracellular compartment, where it serves as a co-factor in a number of biological systems that regulate enzymatic activities and neuromuscular functions. The concentration of cytosolic Mg of about 5×10^{-4} M is very close to the concentration in the extracellular fluid, and both pools are closely regulated by systems that have not been characterized. Serum Mg exists in the ionic state (55%), protein-bound (30%), and complexed (15%). Ionic Mg is the fraction that most closely correlates with Mg-dependent biological actions. Serum total or ionic Mg is not a good estimate of intracellular, soft tissue, or total body Mg content.

Cellular Distribution

Free, ionic cytosolic Mg represents 5–10% of total cellular Mg and is controlled through uptake of Mg by intracellular organelles. Sixty percent of cell Mg is located within the mitochondria where it functions as a cofactor in a number of enzyme systems involved in PO_4 transport, transcription, translation, and ATP use. The transporters and channels involved in maintaining the intracellular distribution of Mg are poorly understood.

Homeostasis

Mg blood levels are regulated largely by the quantitative influx and efflux of Mg across intestine, bone, and kidney rather than an elaborate hormonal system that has evolved for control of Ca. Blood ionic Mg is less potent than $[Ca^{2+}]$ in regulating PTH secretion. Serum Mg levels are regulated primarily in the kidney at the level of renal tubular Mg reabsorption.

Intestinal Absorption

Mg intake is generally adequate, because Mg is a constituent of foods of cellular origin and intake is proportional to total caloric intake. Net intestinal Mg absorption increases in direct proportion to dietary Mg intake. Above 2 mmol (28 mg) per day, Mg absorption exceeds Mg secretion, and Mg balance is positive. During usual Mg intake of 7–30 mmol (168–720 mg/day), fractional Mg absorption averages 35–40%. Net Mg absorption varies with dietary constituents such as P, which forms nonabsorbable complexes with Mg and thereby reduces Mg absorption. Unlike Ca absorption, Mg absorption is not stimulated by $1,25(OH)_2D_3$, and there is no correlation between serum $1,25(OH)_2D_3$ and net Mg absorption. Reduced Mg absorption occurs with diffuse intestinal disease or during chronic laxative abuse.

In both small intestine and colon, absorptive and secretory Mg fluxes have voltage-dependent and voltage-independent components, consistent with both cellular and paracellular pathways. Intestinal luminal Mg concentration drives passive diffusional absorption along the paracellular pathway. Saturable Mg absorption is small compared with the total Mg absorptive flux, indicating a modest cellular, regulated Mg flux.

Renal Handling and Urinary Excretion

Filtration. Ionized and complexed Mg are ~70% of total serum Mg and constitute the ultrafilterable Mg. Urine Mg averages about 24 mmol/day, indicating that ~95% of the GFR is reabsorbed before excretion.

TABLE 3. FACTORS INFLUENCING RENAL Mg REABSORPTION

Glomerular filtration
Increased
Hypermagnesemia
Decreased
Hypomagnesemia
Renal insufficiency
Tubular reabsorption
Increased
ECF volume depletion
Hypomagnesemia
Hypocalcemia
Metabolic alkalosis
Parathyroid hormone
Decreased
ECF volume expansion
Hypermagnesemia
Phosphate depletion
Hypercalcemia
Loop diuretics
Aminoglycoside antibiotics
Cisplantin
Cyclosporin A
Ethanol
Gitelman's syndrome

Reabsorption. In contrast to the 70% reabsorption of filtered Ca in the proximal tubule, this segment reabsorbs only ~15% of the Mg ultrafiltrate. About 70% of Mg is reabsorbed in the cortical TALH and no reabsorption in the medullary TALH. Mg may also stimulate CaR, resulting in decreased Mg reabsorption. Paracellin 1 in the tight junctions also regulates Mg reabsorption. The distal convoluted tubule reabsorbs ~10% of Mg through a transcellular transport process.

Factors Affecting Reabsorption. A number of factors may increase or decrease urine Mg reabsorption (Table 3). ECF volume expansion decreases Mg reabsorption and increases urine Mg because distal portions of the nephron are incapable of reabsorbing Mg. Hypermagnesemia increases urine Mg excretion in part through activation of CaSR. Hypomagnesemia increases TALH Mg reabsorption and decreases urine Mg. Hypercalcemia decreases Mg reabsorption in the proximal tubule and TALH. PTH increases Mg reabsorption, but in primary hyperparathyroidism, the hypercalcemia reduces Mg reabsorption. No other hormones are known to alter Mg reabsorption. Vitamin D and metabolites have no known action on Mg reabsorption. Thiazide diuretics have a minimal effect on Mg excretion. Loop diuretics markedly increase urine Mg excretion. Genetic disorders of renal Mg handling include: Gitelman's syndrome, which is associated with increased urinary Mg excretion because of a mutation of the chlorothiazide-sensitive NaCl co-transporter in the distal convoluted tubule; Bartter's syndrome, which is not associated with changes in serum Mg; and mutations of the CaR, which are associated with defective renal Mg transport.

PHOSPHORUS (PO_4)

Total Body Distribution

About 17,500 mmol (542 g) of PO_4 are found in adult humans (Fig. 6). Eighty-five percent of the total is contained in the hydroxyapatite crystals in bone and 15% is present in soft tissues, with only 0.1% in the extracellular fluids. In soft tissues, PO_4 is in the form of phosphate esters. Only modest

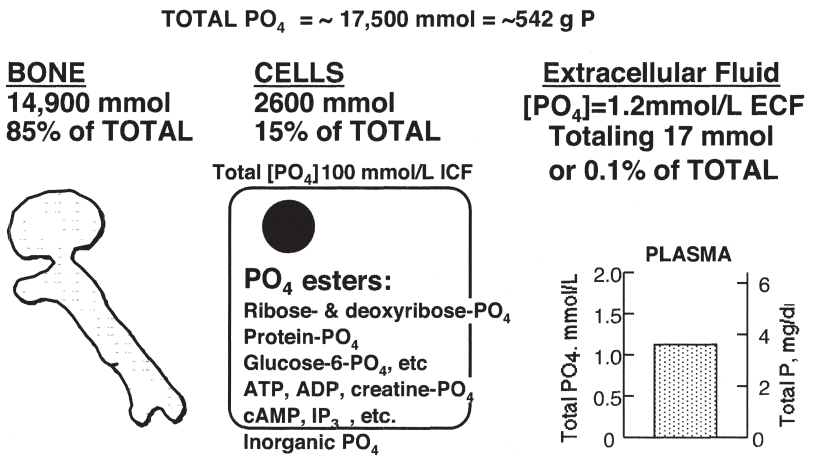

FIG. 6. Phosphate content and distribution in a 70-kg adult.

PO_4 gradients exist across the plasma membrane, with concentration in the extracellular fluids and cytosol of about 10^{-4} and 2×10^{-4} M, respectively. Unlike the tight control of serum Ca, serum PO_4 levels fluctuate widely depending on sex, age, dietary intake, rate of growth, and levels of several hormones. Serum PO_4 is largely determined by the efficiency of reabsorption of filtered PO_4. Serum PO_4 declines in the postprandial state and during intravenous glucose administration through insulin-mediated cellular PO_4 entry. P in serum is mainly in the inorganic form as PO_4 and exists in three fractions: ionic (55%), protein-bound (10%), and complexed to sodium, Ca, and Mg (35%). An adequate PO_4 concentration in serum is required to maintain the Ca $\times$ PO_4 ion product sufficient to support mineralization of bone. Low serum PO_4 levels may create a suboptimal Ca $\times$ PO_4 ion product and impair skeletal mineralization. A pathologically high Ca $\times$ PO_4 product in serum and extracellular fluids promotes ectopic or extraskeletal soft tissue calcification. A detailed discussion of the state of the three fractions of PO_4 in serum and the influence of protein binding and pH are found elsewhere in the primer.

Cellular Distribution

The majority of intracellular PO_4 ion is either bound or exists as inorganic phosphate esters, phospholipids in cell membranes, or phosphorylated intermediate molecules involved in a wide variety of biochemical processes including the generation, storage, and transfer of energy. Cytosolic free PO_4 ion concentration is quite low, whereas mitochondrial PO_4 represents a large proportion of total cellular PO_4, mainly in the form of Ca PO_4 salts.

Intestinal Absorption

PO_4 is found in most all food groups, and PO_4 absorption is directly related to dietary PO_4 intake (Fig. 7). Dietary PO_4 absorption is dependent on both passive transport driven by lumenal PO_4 concentration, which is maximal after a meal, and active, cell-mediated PO_4 transport stimulated by $1,25(OH)_2D_3$. Because PO_4 is a major component of all cells, dietary PO_4 intake is seldom $<$20 mmol (620 mg) per day. Using synthetic diets low in PO_4, metabolic balance studies show that net intestinal PO_4 secretion occurs when diet PO_4 is $<$10 mmol (310 mg) per day. However, when dietary PO_4 intake is within the usual range of 25–60 mmol (775–1860 mg) per day, 60–80% of dietary PO_4 is absorbed. Under conditions of normal dietary PO_4 intake, $1,25(OH)_2D_3$ does not stimulate jejunal PO_4 absorption. However, in patients with vitamin D deficiency or in chronic renal failure with low $1,25(OH)_2D_3$ production, the administration of $1,25(OH)_2D_3$ stimulates net PO_4 absorption. Even in patients with chronic renal failure who have low to undetectable serum $1,25(OH)_2D_3$ levels, there is significant net PO_4 absorption. In fact, the relationship between dietary PO_4 intake and net PO_4 absorption is the same in patients with chronic renal failure and in normal subjects. PO_4 absorption is reduced with diffuse small intestinal disease such as malabsorption syndromes.

Transepithelial PO_4 transport must overcome existing electrochemical gradients as the ion moves from intestinal lumen into the enterocyte. Entry across the brush border is driven either by an energy-dependent transport process or a secondary active transport process coupled to the flux of another ion such as Na. The linear increase in PO_4 uptake with luminal PO_4 and Na support the importance of Na-PO_4 co-transport across the intestinal brush border. $1,25(OH)_2D_3$ stimulates net intestinal PO_4 absorption through enhanced cellular brush border PO_4 uptake. The uptake process is saturable with an affinity coefficient of 1.0 mM. Thus, at luminal PO_4 concentrations $>$1.0 mM, diffusional PO_4 absorption predominates, but when lumen PO_4 concentration is low, as during low diet PO_4 intake, the transcellular mechanism is active. The saturable active trans-

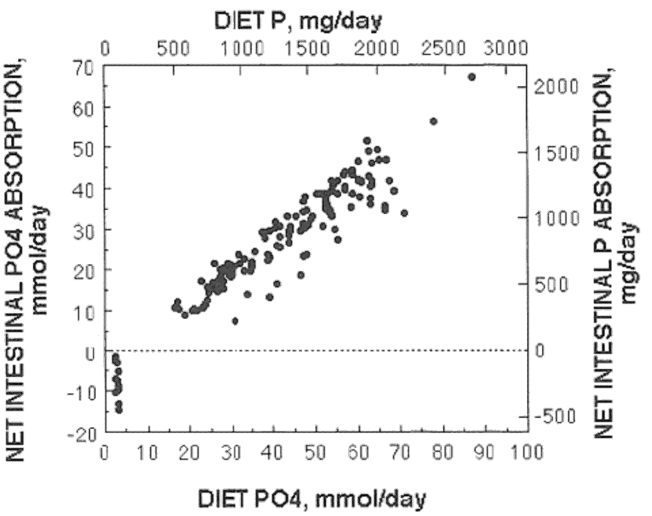

FIG. 7. Net intestinal PO_4 absorption in relation to dietary P intake. Net intestinal P (mmol/day) = $-5.4 + 0.77 \times$ dietary P (mmol/day); $r = 0.95$. Adapted with permission from top panel of Fig. 42. In: Coe FL, Favus, MJ, Pak CYC, Parks JH, Preminger GM (eds.) Kidney Stones: Medical and Surgical Management, 1st ed. Lippincott-Raven, Philadelphia, PA, USA, 1996, p. 281.

port mechanism is present in proximal duodenum, jejunum, and to a lesser extent, in distal ileum. Net PO_4 absorption does not occur in colon.

Renal PO_4 Handling and Urinary Excretion

Filtration. Approximately 85% of serum PO_4 is ultrafilterable, and urine PO_4 excretion is ~25–33 mmol (750–1000 mg) per day. Thus, ~12.5% of glomerular filtrate is excreted in the urine.

Reabsorption. Eighty-five percent of PO_4 reabsorption occurs in the proximal tubule. The rate-limiting step in PO_4 reabsorption is located in the apical domain of the proximal tubule cells, which is also the site of active Na-PO_4 co-transport. The transporter moves PO_4 against trans-brush border electrochemical PO_4 gradients and follows a transcellular pathway that is dependent on low intracellular Na concentration. Three genes encode related Na gradient–dependent phosphate transporters (Npt1–Npt3), and the apical brush border membrane Npt2 accounts for ~85% of proximal tubule PO_4 reabsorption. Npt2 but not Npt1 is regulated, and a major regulator of Npt2 is FGF-23. Elevated levels of FGF-23 have been found in patients with disorders of hypophosphatemia and renal PO_4 wasting including X-linked hypophosphatemic rickets (XLH), tumor-induced osteomalacia, and autosomal dominant hypophosphatemic rickets. Other so-called phosphotonins have been identified that may also regulate Npt2. Beyond the proximal tubule, a small fraction of PO_4 reabsorption occurs in the distal convoluted tubule. Tubular maximum for the reabsorption of PO_4 is regulated and is approximately equal to the normal amount of PO_4 filtered by the glomerulus. Thus, any appreciable increase in filtered PO_4 increases urinary PO_4 excretion.

Factors Affecting Reabsorption. Dietary PO_4 intake, PTH, and FGF-23 are the major regulators of Na-dependent PO_4 reabsorption (Table 4) that determine the maximal capacity of the kidney to reabsorb filtered PO_4. Low PO_4 intake stimulates reabsorption, whereas high PO_4 intake inhibits reabsorption and increases urine PO_4. These alterations in proximal tubule PO_4 reabsorption over a range of PO_4 intakes are independent of changes in PTH, level of serum Ca, or ECF volume. Changes in PO_4 intake induce an inverse modulation in the maximal rate of the brush border Npt2 co-transporter within hours and thereby alter the level of PO_4 reabsorption. The effects of hypercalcemia and hypocalcemia are to decrease and increase renal PO_4 reabsorption, respectively. Part of the renal response to hypercalcemia is the associated decrease in GFR and therefore filtered PO_4. ECF volume expansion decreases proximal tubule Na and PO_4 reabsorption. PTH is a phosphaturic hormone and the principle regulator of renal PO_4 reabsorption and excretion. PTH decreases proximal tubule PO_4 reabsorption through suppression of the proximal tubule brush border Npt2 co-transporter. $1,25(OH)_2D_3$ increases intestinal PO_4 absorption and decreases renal PO_4 reabsorption. Insulin increases renal PO_4 reabsorption, and glucocorticoids and glucagon decrease renal PO_4 reabsorption. As discussed elsewhere, FGF-23 is a major circulating phosphaturic factor (phosphotonin) that is detectable in the circulation of normal subjects and in some hypophosphatemic disorders. Serum FGF-23 levels are positively correlated with serum PO_4 and suggest a role in normal physiologic control of renal PO_4 reabsorption.

TABLE 4. FACTORS INFLUENCING RENAL PO_4 REABSORPTION

Glomerular filtration
Increased
Hyperphosphatemia
Mild hypercalcemia
Decreased
Hypophosphatemia
Renal insufficiency
Moderate hypercalcemia
Tubular reabsorption
Increased
Dietary PO_4 deprivation
Hypercalcemia
ECF volume depletion
Chronic metabolic alkalosis
Decreased
PO_4 excess
Hypocalcemia
Acute metabolic alkalosis
Chronic metabolic acidosis
Parathyroid hormone
Parathyroid hormone related protein
FGF-23
Calcitriol
Thiazide diuretics
X-linked hypophosphatemic rickets
Autosomal dominant hypophosphatemic rickets
Tumor-induced osteomalacia

MINERAL BALANCE AND ADAPTATION TO CHANGING REQUIREMENTS

Mineral balance refers to the state of retention or loss of Ca (Figs. 2 and 3), Mg, and PO_4 (Fig. 8) with respect to the external environment. Adaptation refers to adjustments in the mineral homeostatic system to meet mineral requirements and return the system to neutral balance. With respect to Ca, the homeostatic system is sufficiently flexible to maintain blood ionized Ca within the normal range during wide variations in dietary Ca intake and changing rates of bone mineralization. Thus, adaptations of the Ca homeostatic system occur while blood Ca remains stable. External mineral balance is determined by feeding subjects constant diets analyzed for Ca, Mg, and PO_4. Subjects are adapted to the diet for 7–10 days, especially if the quantity of the ions in the test diet differs significantly from a subject's usual intake. After a period of adaptation, a balance period of at least 6 days is conducted, during which time the diet is continued and all fecal and urine passed are analyzed for the ions. A nonabsorbable marker of fecal collection is administered with each meal to assess completeness of fecal collection. Dietary intake minus average daily fecal excretion during the balance period estimates net intestinal absorption of Ca, Mg, and PO_4 (Figs. 2, 3, and 8). Several examples of physiologic adaptation or pathological alteration in Ca, Mg, and PO_4 balances are presented below.

Neutral Balance

In neutral mineral balance, mineral ion absorptions match the sum of urinary and fecal mineral losses (Figs. 3 and 8). Neutral balance is found in adult men <65 years of age and in premenopausal, nonpregnant women after reaching peak bone mass.

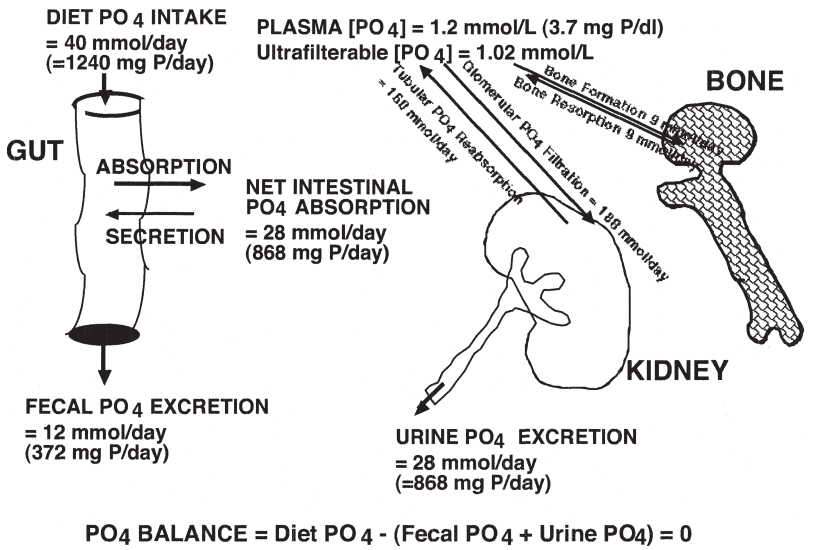

FIG. 8. Daily PO_4 turnover in a 70-kg adult

Positive Balance

In positive balance, mineral absorption and retention exceed fecal and urine mineral losses. Positive balance of Ca, Mg, and PO_4 occurs during skeletal growth during childhood and adolescence and during pregnancy and lactation. Adaptation such as during skeletal growth is characterized by increased efficiency of intestinal mineral absorption with greater influx of ions into the ECF space; reduction of urine mineral ion losses through increased renal tubule reabsorption to return mineral into the ECF space; and the resultant increased serum mineral ion levels that enhance delivery and deposition at skeletal mineralization sites. Increased mineral ion transport is stimulated indirectly by PTH and by direct actions of $1,25(OH)_2D_3$ through insertion of ion channels and transporters into epithelial membranes which enhance transepithelial fluxes.

Negative Balance

Negative mineral ion balance is defined by mineral loss (fecal plus urine) exceeding mineral retention and may be associated with clinically significant bone loss. Negative mineral ion balance with bone loss occurs in postmenopausal women and during estrogen deficiency at any age. Pregnancy is associated with negative mineral balance to the extent that minerals are transferred from the mother's skeleton across the placenta to the rapidly mineralizing fetal skeleton. However, balance studies performed on pregnant women reveal positive mineral balance because of the retention of mineral ions by the combined maternal-fetal unit. Bone loss and negative mineral ion balance occur during chronic glucocorticoid excess, hyperthyroidism, and vitamin D deficiency. In all three examples, intestinal mineral ion absorption is reduced, mineral fluxes into bone are exceeded by bone mineral ion efflux, and urinary ion excretion may be excessive (glucocorticoid excess and hyperthyroidism) or low (vitamin D deficiency).

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Chapter 14. Gonadal Steroids and Receptors

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INTRODUCTION

Since steroid hormones were discovered in the early 1900s, studies of their molecular actions have extended from the whole body, to the target tissues, to specific functions within those target tissues, to the specific cells, and finally to specific genes and their protein products. It is well accepted that sex steroids and other steroid family members play an important role in bone cell metabolism, including the regulation of osteoblast and osteoclast activities and reinforcing the coupling between these cells through paracrine factors. Gonadal steroids act on the skeleton to maintain proper bone homeostasis to prevent bone loss. The reduction of systemic estrogen secretion in postmenopausal women, for example, is primarily responsible for type I osteoporosis, a disease characterized by the rapid phase bone loss during the first 5–10 years after menopause. Estrogen replacement therapy reinstates the homeostasis between the osteoblasts and osteoclasts and prevents bone loss, thereby supporting the role of estrogen deficiency in osteoporosis. This chapter presents a brief overview of the structure and function of gonadal (sex) steroid hormones, their receptors, receptor co-regulators, and the mechanisms by which they regulate gene expression and cell function. Reviews, instead of original papers, are often used in this chapter to limit the numbers of references and to give further direction to the readers for each of the areas discussed in this chapter.

STEROID HORMONES

There are three categories of steroid hormones: glucocorticoids, mineralocorticoids, and the gonadal (sex) steroids (estrogens, androgens, and progesterone), each with different functions in the human body. Sex steroids are synthesized in response to signals from the brain. Certain signals from the central nervous system initiate a stimulus from the hypothalamus to the pituitary gland, which releases peptide hormones that target the reproductive organs. These peptide hormones, luteinizing hormone, and follicle-stimulating hormone, stimulate the synthesis of progesterone and estrogens in the female ovaries and testosterone in the male testes. Comprehensive reviews on these processes are available.[1–5] Traditionally, sex steroids were thought to only regulate activity in reproductive organs, but the discovery of steroid hormone receptors throughout the body has implicated varied patterns of functionality in many other tissues.

Biosynthesis of Steroid Hormones

The synthesis of steroid hormones from cholesterol involves pathways with 10 or more enzymes (Fig. 1A).[4,5] The level of steroid hormones in the bloodstream is primarily controlled by its rate of synthesis, because little of the steroid reserves are maintained. The increase of these hormones in the serum takes from hours to days, so cellular responses are delayed but last longer than the effects mediated by the peptide hormones. In ovulating women and during pregnancy, progesterone is secreted by the ovaries. Progesterone not only exhibits biological activity, but as shown in Fig. 1A, also serves as a precursor for all other steroid hormones. Estrone and estradiol are formed from androstenedione and testosterone, respectively (Fig. 1A). This reaction is mediated by the enzyme aromatase, a cytochrome P-450 enzyme present in the ovary, as well as the testis, adipocytes, and osteoblast/osteocyte cells. Estradiol and estrone are in reversible equilibrium because of hepatic and intestinal 17β-hydroxysteroid dehydrogenases. The major circulating estrogen in postmenopausal women is estrone, which, in turn, follows two main pathways of metabolism, resulting in 16α-hydroxyestrone (active) and 2-hydroxyestrone (inactive/weak).[6] The balance in the ratio of these hydroxylated estrones along with the local production of estrogens by aromatase have been implicated as playing roles in several disease states, including breast cancer, osteoporosis, systemic lupus erythematosis, and liver cirrhosis.[7]

The steroidogenic pathway is essentially the same in the testes and in the ovaries, with the exception that testosterone is the major secretory product, although small amounts of estradiol are also secreted.[5] Testosterone is converted to more active metabolites in target tissues, such as gonads, brain, and bone. This modification is accomplished by two enzymes: 5α-reductase and aromatase.[5] As depicted in Fig. 1A, the 5α-reductase irreversibly converts testosterone to dihydroxytestosterone, which cannot be aromatized to estrogens. Conversely, aromatase irreversibly converts testosterone into estrogenic molecules.

Transport Through the Bloodstream

The major sex steroids in circulation are androgens and estrogens (estradiol and estrone). Because their chemical structure makes them fat soluble, most are bound to specific carrier proteins (e.g., serum [or sex hormone] binding globulins [SBGs]) for transportation through the bloodstream to hormone receptors that reside in target cells. Only 1–3% of the total circulating biologically active sex steroids are free in solution. Both the free steroid and the albumin-bound fraction (35–55%) can enter target tissues, thereby representing the "bioavailable" steroid pool. The remaining fraction, bound to the SBGs, is unable to enter the cells. The sex steroids enter all cells by simple diffusion through the cell membrane and subsequently bind to specific receptor molecules to regulate gene transcription.

Selective Estrogen Receptor Modulators

Steroid analogs, called selective receptor modulators (SRMs), have been developed to elicit tissue-specific rather than systemic effects, avoiding common side effects of steroid replacement therapy. Selective estrogen receptor (ER) modulators (SERMs) bind to estrogen receptors as a ligand, but act either as agonists or antagonists, depending on the cell/tissue-type (Fig. 1B). For example, the SERM, tamoxifen, is an antagonist in reproductive tissues with little effect on bone, and raloxifene is an agonist in bone with little effect in reproductive tissues. Other compounds (e.g., diethylstilbestrol) are not steroids but still bind with relative high affinity to the ER to mimic estrogen action in reproductive and bone tissues. The synthetic steroid, ICI 182,780, a pure antiestrogen, binds to the ER and as a pure antagonist blocking all ER activity. Recent studies have shown that SERMs exert many of their actions by regulating a unique set of genes or differentially regulating the same genes as does estrogen.[8,9] The progesterone antagonist RU 486, along with the androgen receptor inhibitor, flutamide

The authors have reported no conflicts of interest.

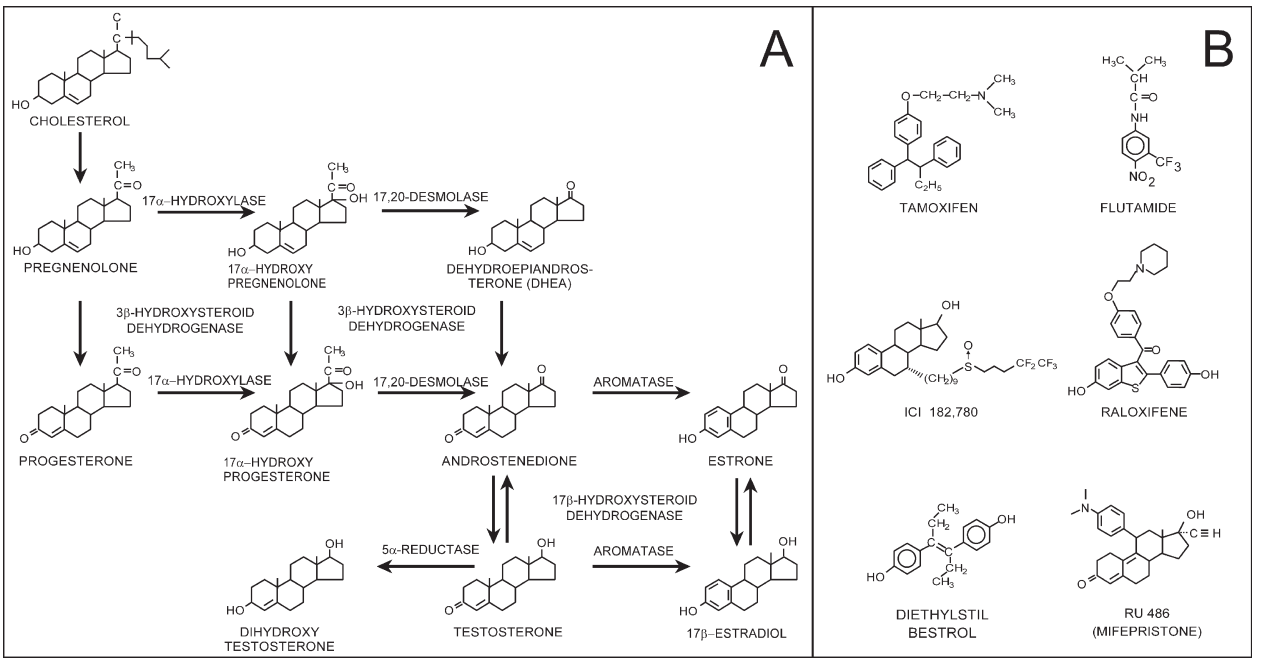

FIG. 1. (A) Pathways of steroid hormones biosynthesis from cholesterol. The initiation of steroid hormone synthesis involves the hydrolysis of cholesterol esters and the uptake of cholesterol into mitochondria of cells in the target organ. Dehydrogenation of pregnenolone produces progesterone, which serves as a precursor molecule for the generation of all other gonadal steroid hormones. (B) Chemical structures of common steroid analogs. [Adapted from Khosla et al. 1999 Dynamics of bone cartilage metabolism. In: Seibel M, Robins S, Bilezikian J (eds). Sex Steroid Effects on Bone Metabolism. pp. 233–245, with permission from Elsevier.[28]]

(aSARM), functions in a similar manner to SERMs. Further studies with SERM analogs, including their effects on steroid receptor structures and co-activator recruitment, are providing new alternatives to steroid replacement therapy.[3,10,11]

STEROID HORMONE RECEPTORS

Steroid hormones generate their intracellular signaling by binding to steroid-specific proteins called receptors. There are three steps in the general mechanism of action for steroid receptors: (1) binding of the steroid ligand to the receptor in the nucleus, (2) translocation of the steroid receptor complex to a specific site on the DNA, and (3) the regulation of gene transcription. Each type of steroid receptor is activated by a unique steroidal ligand. As shown in Fig. 2, steroid receptor proteins have multiple "domains," each of which has specific functions. All steroid receptor species share significant homology in terms of peptide sequence and functional domains. There are currently two sites, located in domains I and IV, identified as having transcriptional activation functions (TAFs). Additionally, a sequence of basic amino acids near the second zinc finger in domain III comprises a nuclear localization signal, allowing for the interaction of the receptor with nuclear transport proteins and subsequent entry into the nucleus. Other nuclear localization signals have been identified in the hormone-binding domain (IV) and are hormone-dependent.[12,13] As shown in Fig. 2B, the receptor domains have been further divided into six domains (A/B, C, D, E, and F) based on molecular structure and functions.

It should be mentioned that all species of sex steroid receptors, except for the androgen receptor, have two receptor isoforms (species) coded from the same or different genes (see Fig. 2B).[14] Furthermore, some isoforms have been shown to include several species or "variants" of each isoform.[15,16] Traditionally, it was thought that one isoform or splice variant of a particular nuclear receptor acted as the primary mediator of gene expression, whereas the other variants antagonized the primary receptors effects. Recent data, however, suggest that ERα and ERβ regulate mainly distinct sets of genes and unique effects on cellular proliferation and other pathways.[8,9]

Heat Shock Proteins as Steroid Receptor Chaperones

In their inactive state, steroid receptors exist as part of a large complex in association with other non–steroid-binding proteins within cells not exposed to the steroid. Many of these proteins are members of the heat shock protein family that are classified by size (e.g., hsp90, hsp70, hsp60, hsp40, and hsp27) and act as molecular chaperones that bind to client proteins (e.g., receptors). Heat shock proteins assist in protein folding, intracellular transport, protein repair, and degradation of damaged proteins.[17,18] Overall, heat shock proteins are required for the activation and functionality of many proteins, including steroid receptors.

Receptor Activation

Each receptor reversibly binds its respective steroid with high affinity and specificity. However, few steroid receptors exhibit absolute ligand specificity (e.g., gonadal steroids can bind to more than one class of receptor) if the steroid ligands are at sufficiently high concentrations that encourages lower affinity interactions. This lack of steroid specificity among receptors is not unexpected in view of the significant homology among their ligand binding domains. The primary effect of ligand binding to domain IV is to "activate" the receptor molecule by inducing a conformational change in the whole protein, so that it can interact with DNA. This activation, as a result of ligand binding, involves the release of the chaperones (described above) and a conformational change in the receptor molecule, especially domain IV. This overall ligand-dependent

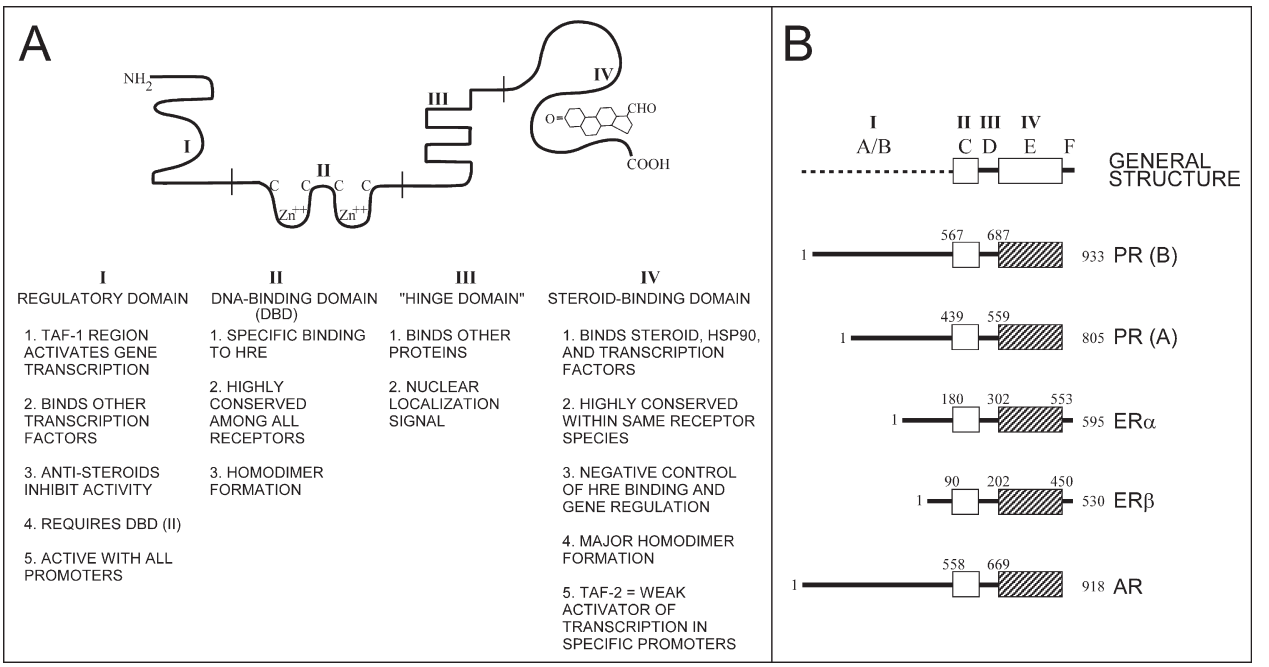

FIG. 2. (A) Structure and functional domains of steroid hormone receptors. Proceeding in the N-terminal to C-terminal direction, the receptors contain a variable domain (I or A/B), thought to be involved in cell type–specific regulation of gene transcription, a DNA binding domain (II or C) of 66–68 amino acids, which shows a high degree of homology among members of the steroid receptor family, a "hinge" domain (III or D), a steroid binding domain (IV or E), showing some homology, and variable regions (V or F) with little homology that somehow contribute to optimal function of the receptor. (B) Structural homology among sex steroid receptor family members. The sex steroid receptors range in size from 530 amino acids for the estrogen receptor to 933 amino acids for the progesterone receptor. Human progesterone receptors exist in two isoforms, A and B, generated by the same gene through differential promoters. The estrogen receptor also exists in two forms (ERα and ERβ); however, they arise from different genes, each with unique domains that allow for tissue- and ligand-specific functions. TAF, transcriptional activation function; DBD, DNA binding domain; HRE, hormone response element. [Adapted from Oursler et al. 1996 Osteoporosis. In: Marcus R, Feldman D, Kelsey J (eds.) Regulation of Bone Cell Function by Gonadal Steroids. pp. 237–260, with permission from Elsevier.[36]]

conformational change seems to be conserved across steroid family members.

CONTROL OF GENE EXPRESSION

The mechanism by which steroid hormone receptors exert their effects on target cells is 2-fold (Fig. 3). Initially, steroid hormones can act in a nongenomic manner through binding to steroid receptors associated with the plasma membrane. The resulting steroid receptor activation initiates a signal transduction cascade modulated by various kinase pathways that eventually alter the activities of select transcription factors. The second type of steroid action involves the better understood genomic pathway, by which steroid receptors in the cytoplasm and/or nucleus are activated after ligand binding and subsequently act as transcription factors to regulate gene expression. In the classical or canonical genomic pathway, ligand-bound steroid receptors bind to specific DNA sequences called hormone response elements (HREs), which are unique for each steroid receptor. In the non-canonical genomic pathway, activated receptors bind indirectly to the DNA through specific transcription factors (e.g., activating protein-1 [AP-1], signal protein-1 [SP-1]; see Fig. 3). Regulation of steroid receptor activity is further controlled by association with receptor co-regulator proteins (co-activators/co-repressors). Co-regulators bind to steroid receptors before and after receptor/DNA interactions and act with the general transcriptional machinery to either enhance or inhibit gene expression (Fig. 3).[14,19]

Genomic Pathway

Receptor Binding to DNA Element. The DNA binding domain of the receptor contains two "zinc fingers" (looped structures involving chelated metal ions) that are responsible for the binding to the HREs of target genes in what is termed the canonical pathway. A characteristic hexanucleotide inverted palindromic repeat allows the receptor to bind the DNA as a dimer. The orientation, sequence, and space between the hexanucleotide repeats are unique for each steroid hormone.[20] The receptors bound to the DNA through transcription factors (non-canonical pathway) also bind as dimers.

General Co-Regulator Function—Co-Activators and Co-Repressors. Steroid hormone receptor co-activators are involved in enhancing the transcriptional signal of the steroid hormone receptors after ligand binding.[11] Steroid receptor co-activator-1 (SRC1), the founding member of the SRC family (also called the p160 family), was originally identified as an interacting protein with the progesterone receptor (PR) ligand binding domain (LBD).[21] Further analysis showed that SRC1 exhibits transcriptional co-activation properties not only with the PR but also with nearly all type I nuclear hormone receptors. Two additional members of the p160 family of co-activators, SRC2 and SRC3, were also identified which interact with and co-activate numerous nuclear hormone receptors. The p160 family of proteins mediate the interactions with steroid hormone receptors through a centrally located receptor interaction domain (RID) of the p160 protein, which contains three α-helical LXXLL motifs necessary for interaction with steroid hormone receptors.[10,11,14] Binding of ligand to the receptor induces a conformational change that allows association of the RID domain with the LBD/AF-2 function of the steroid hormone receptor. Mutation or deletion of the SRC RID domains disrupts physical interaction with the steroid receptor LBD and

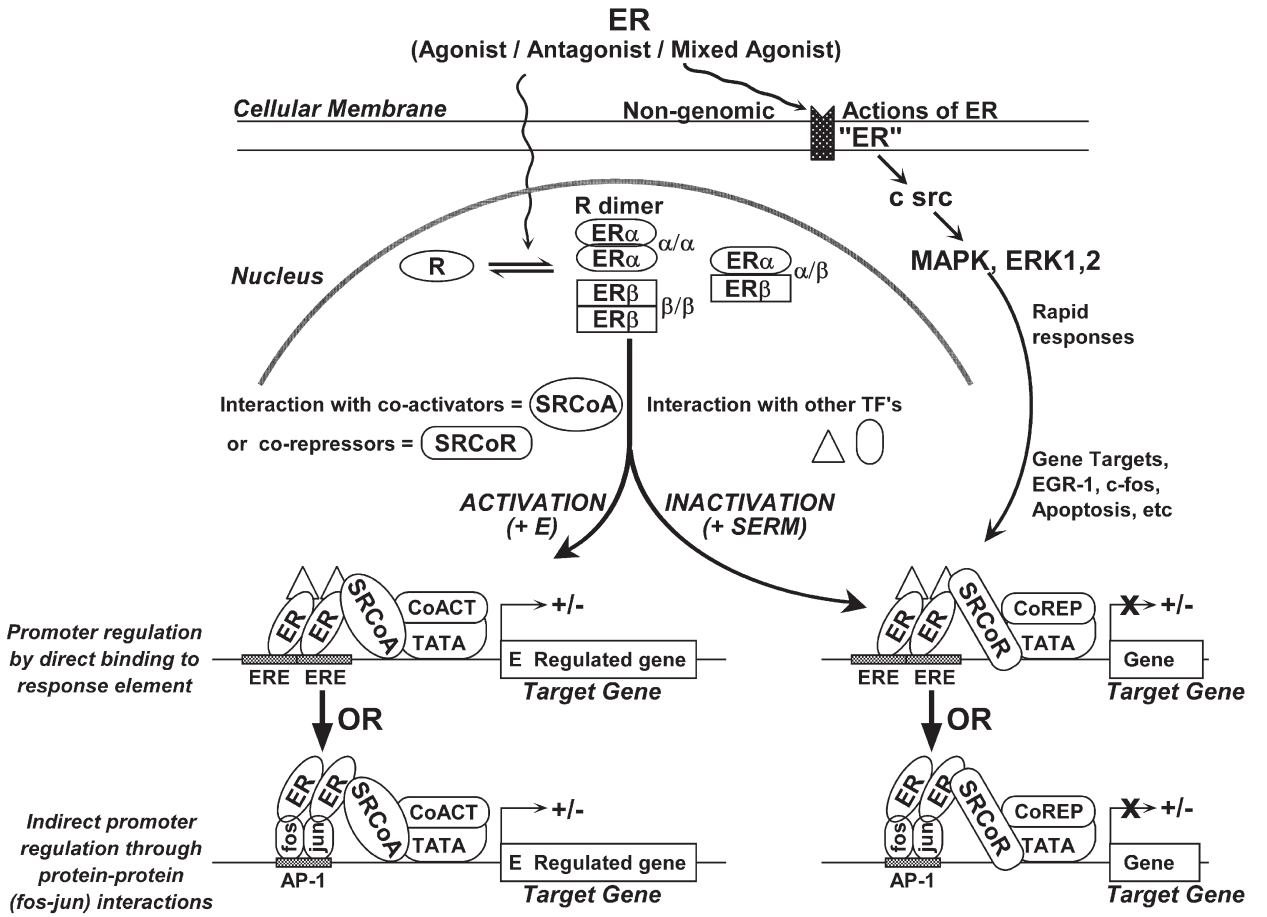

FIG. 3. Overall mechanism of action of steroid (estrogen) receptors. The inactive receptor is complexed to several heat-shock proteins (hsp). On binding of the steroid hormone, a conformational change in the receptor occurs causing the receptor to become "activated" and function as a transcriptional regulator in the genomic pathway. Alternatively, steroidal/nonsteroidal activation of a plasma membrane–associated receptor (steroid receptor or unknown membrane variant) can lead to rapid downstream cellular responses through nongenomic pathways. In the genomic pathway, the two isoforms of most sex steroid receptors (e.g., ERα or ERβ) form homo- or heterodimers and form large complexes with various co-regulators (co-activators or co-repressors), enabling the receptor to associate with targeted genes and regulate their expression. The binding of an agonist ligand to either receptor isoform causes a conformational change to occur in the ligand binding domain (LBD) of the steroid receptor followed by recruitment of primary co-activators (SRCoA), such as the SRC family of co-activators. This in turn, recruits other transcription factors (sometimes called "secondary" co-activators), such as p300/CBP, and histone acetylases to open the chromatin DNA, allowing for recruitment of the basal transcription complex and culminating in a productive transcriptional response. Some co-activators recruit HAT to destabilize histones and nucleosomes, whereas other co-activators contain this activity intrinsically. Binding of an antagonist ligand to the receptor, on the other hand, produces a conformational change in the LBD of the steroid receptor which recruits specific steroid receptor co-repressors (SRCoR) that cannot recruit the transcription co-activators necessary for a productive transcriptional response. These co-repressors recruit histone deacetylases (HDAC), which re-establishes the histone–DNA complexes and nucleosome structure and blocking DNA for transcription.

thus abolishes the transcriptional co-activation potential of the SRC molecule and the steroid receptor.[10,11,14] The p160 family of co-activators contains intrinsic transcriptional activation domains that contribute to the overall transcriptional activation elicited by the ligand-bound steroid hormone receptor. These domains function to recruit other molecules involved in activating transcription such as CBP/p300 (see Fig. 3). One interesting domain found in both SRC1 and SRC3 is the histone acetyltransferase (HAT) domain. This domain functions to modulate a condensed chromatin structure into a conformation permissive to transcriptional activation. HAT activity serves to transfer acetyl groups to specific lysines in histones and is thought to "loosen" the histone grip on DNA, facilitating the entry of other transcription factors (or the basal transcriptional machinery itself) to activate transcription. Thus, the various co-activators serve numerous functions in the processes of transcriptional activation.

Transcriptional repression by steroid hormone receptors is mediated by the recruitment of co-repressors that function by competing with co-activators for the ligand-bound LBD of steroid receptors. The co-repressor, REA (repressor of estrogen action), functions in this manner by directly competing with SRC1 in binding to the LBD.[22] Other transcriptional co-repressors bind the LBD either in the absence of ligand or in the presence of steroid receptor antagonists such as tamoxifen or ICI 182,780. The structure of co-repressors in many ways mirrors that of co-activators. Certain co-repressors contain histone deactylation interacting domains that seem to antagonize transcriptional activation by recruiting factors involved in histone deactylation, called HDACs (protein complexes that tightly pack DNA, rendering DNA elements inaccessible to transcription factors).[10,11,14] Thus, the activities of co-repressors and co-activators target similar processes in opposing manners (e.g., HAT activity versus HDAC activity). The regulation of co-activator and co-repressor function is a major component in determining the activation state of a gene. Figure 3 outlines the sequence of events that may occur when either a co-activator or a co-repressor is bound to the steroid receptor.

However, it must be noted that nuclear receptor activation is not a static process. Studies involving the promoter region of the E2-dependent gene pS2, showed that unliganded ER cycles on and off the promoter until bound by E2. Steroid binding results in a conformational change of the receptor, stabilizing the DNA/protein interaction, thus allowing for RNA polymerase and nuclear co-regulator association.[23] This process is highly transient and cyclic with receptors and co-regulators binding, dissociating, and rebinding with a 20-minute periodicity.[23]

Nongenomic Pathway. There is a growing body of evidence that steroids can alter cell metabolism by nongenomic effects (i.e., without direct interaction with DNA or transcription factor DNA complexes). More recent studies[24–26] have confirmed previous data showing that steroid hormones can act through nongenomic signaling pathways. These effects have been characterized by rapid responses ranging from seconds to minutes, involving steroid interaction with membrane receptors or steroid receptor activation in the absence of ligand. Nongenomic estrogen effects signal through the activation of cell surface receptors, leading to alterations in cAMP levels, calcium influx, and direct channel gating. Immediate early responses by estrogen can be mediated by ERα through the direct interaction with Src tyrosine kinase, leading to the activation of multiple signaling cascades, including mitogen-activated protein kinase (MAPK).[27] Mechanistically, covalent modifications of the steroid receptor, such as phosphorylation, are thought to be responsible for the activation of nuclear receptors by nonsteroid effectors.

PHYSIOLOGY OF STEROID EFFECTS ON BONE

The two major types of cells in bone that are responsible for the maintenance of normal bone density are osteoblasts (OBs) and osteoclasts (OCLs).[28–30] Normal bone remodeling processes involve bone resorption by OCLs and bone formation by OBs, which are tightly coupled to prevent a net loss of bone mass. Many factors that influence bone resorption can either act directly on the OCLs or indirectly through the OBs. Some of these factors are local regulators, including interleukins, TNF, prostaglandins, and TGF-β, or systemic factors, including PTH, vitamin D_3, calcitonin, glucocorticoids, and the sex steroid hormones.

Estrogens

Estrogen acts as a potent anabolic steroid regarding bone mass, reducing bone resorption indirectly by inhibiting osteoclastogenesis and directly by inhibiting OCL function. In postmenopausal women, there is an increase in bone remodeling activity in which resorption is no longer coupled with bone formation. After menopause, women lose bone mass at an average rate of 3% annually compared with 0.5–1.0% for similarly aged men.[31] Estrogen deficiency is recognized as the most important factor in the pathogenesis of postmenopausal bone loss, because estrogen replacement therapy has been shown to be effective in preventing and treating osteoporosis. The identification of estrogen receptors in human OBs and OCLs implicated estrogen as a direct effector on these bone cells, as opposed to previous theories that other calcitropic hormones were the primary mediators of the skeletal effects of estrogen deficiency.[28–30] Estrogens have recently been shown to play an important role in the selected physiology of men.[32] The strong influence of the local production of estrogens by the aromatase-cytochrome p450 enzyme in men and postmenopausal women are becoming more apparent.[7] Many of the late effects of steroid receptors are generated as secondary effects through the steroid regulated production of growth factors/cytokines, which in turn, can affect these target cells.

Progesterone

At menopause, there is a decrease in circulating levels of progesterone as well as estrogens,[6] which implicates progesterone in postmenopausal bone loss. However, in contrast to the abundance of work on the effects of estrogens on bone metabolism, there is little evidence of direct effects of progesterone on bone.[33] Clinical studies suggest that the effects of progesterone treatment on postmenopausal women are similar to those of estrogen, but combined estrogen and progesterone treatment has effects that differ from those of treatment with either steroid alone. Because of the adverse side effects of estrogen-alone therapy, alternative therapies, such as the combined estrogen and progesterone treatments, are being pursued. Although estrogen is usually required to induce their synthesis, progesterone receptors have been identified in human OB cells, so progesterone could exert direct effects on bone through its own receptor. Another possible mechanism for progesterone action is through interaction with glucocorticoid receptors. Progesterone can displace glucocorticoids from their receptors and vice versa.[34] Progesterone usually does not activate the glucocorticoid receptor and thus often blocks glucocorticoid responses.

Androgens

Like estrogens, androgens influence bone development and metabolism with dramatic clinical manifestations. Decreased androgen levels have been linked to lower bone density in men, and there is a strong correlation between hypogonadism in elderly men and hip fracture and spinal osteoporosis. Clinical studies also show that treatment of osteoporosis with androgens is effective in increasing bone density in both men and women. However, newer studies suggest that estrogens may play a greater (or equivalent) role than androgens in the male skeleton.[32] Human bone cells have androgen receptor concentrations similar to estrogen receptors and 5α-reductase and aromatase activity for the conversion of testosterone to dihydroxytestosterone and estrogen, respectively. Androgens decrease bone resorption by acting directly on human OCL cells. In addition, androgens also regulate the production of a number of bone-resorbing factors by mature OB or by marrow stromal cells, which contain OB progenitor cells. As with progesterone, further studies are needed to determine the complete role of androgens on bone metabolism.[35]

CONCLUSIONS

Steroid receptors act as cyto-nuclear transcription factors, whose structures are altered by ligand binding, such that the transcription activating domains of the receptor molecule are free to regulate gene expression. The binding of steroid analogs to these receptors can alter the receptor structure, thereby modulating which nuclear co-regulators are bound to the receptor and which TAF region is active. The particular type and relative amounts of steroid receptors in a cell determines the response as much as the circulating hormone levels (e.g., the ratio of ERα and ERβ in a given cell will significantly modulate the response to estrogen). The interaction of steroids with membrane receptors and the activation of steroid receptors in the absence of ligand through phosphorylation events can lead to alternative biological and physiological responses. Additionally, the ratio and presence or absence of specific nuclear co-regulators in a given cell or tissue type may alter steroid-dependent gene expression patterns. These molecular discov-

eries are aiding in the explanation of physiological actions of steroid hormones and their select steroid receptor modulators.

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Chapter 15. Parathyroid Hormone: Synthesis, Secretion, and Action

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INTRODUCTION

Parathyroid hormone (PTH), produced by the chief cells of the parathyroid gland, plays a central role in calcium homeostasis through its actions on bone, kidney, and, indirectly through stimulating the 1-hydroxylation of 25-hydroxyvitamin D_3, intestine. The three key physiological regulators of PTH secretion and synthesis and parathyroid cellular proliferation are extracellular calcium and phosphate ions and 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$]. In response to decrements in extracellular calcium, increases in extracellular phosphate and/or reductions in $1,25(OH)_2D_3$, the parathyroid chief cell secretes and synthesizes more PTH in the short term and undergoes cellular proliferation in the longer term. These three actions will increase blood calcium, reduce blood phosphate, and increase renal synthesis of $1,25(OH)_2D_3$, thereby restoring these physiological perturbations to or toward normal. The effects of PTH are mediated by a G protein–coupled PTH/PTH-related protein (PTHrP) receptor. The actions of extracellular calcium on parathyroid function are also mediated by a G protein–coupled receptor, the extracellular calcium-sensing receptor (CaSR), that also modulates the actions of PTH on the kidney. $1,25(OH)_2D_3$, in contrast, acts through a nuclear transcription factor, the vitamin D receptor (VDR), and the mechanism(s) underlying phosphate sensing are unknown. For the CaSR, PTH receptor, and VDR (discussed elsewhere), studies of naturally occurring mutations in humans as well as genetically modified mice have greatly clarified the functional roles of these receptors. In the case of the CaSR, drugs are now available that activate or inhibit the receptor and are already approved or in clinical trials for use in humans.

PTH and the active form of vitamin D, $1,25(OH)_2D_3$, are the principal regulators of calcium homeostasis in humans and most likely all terrestrial vertebrates.[1,2] In bone, PTH stimulates the release of calcium and phosphate, and in the kidney, it promotes the reabsorption of calcium and inhibits that of phosphate. Furthermore, PTH increases the activity of the renal 1-α-hydroxylase, thereby enhancing the synthesis of $1,25(OH)_2D_3$, which in turn increases the intestinal absorption of calcium and phosphate. As a result of these PTH-dependent actions, blood calcium concentration rises and blood phosphate concentration declines. The extracellular calcium concentration is the most important physiological regulator of the minute-to-minute secretion of PTH. A rise in blood calcium concentration decreases PTH secretion, whereas a reduction in blood calcium increases PTH release. $1,25(OH)_2D_3$ and low phosphate, as well as an increase in calcium, all act to decrease the synthesis of PTH. The mutual regulatory interactions of PTH, calcium, $1,25(OH)_2D_3$, and phosphate can thus maintain the blood calcium level constant, even in the presence of significant fluctuations in dietary calcium, bone metabolism, or renal function. In this chapter, we shall review the structure and biosynthesis of PTH, the regulation of its secretion, particularly by extracellular ($Ca^{2+}{}_o$) acting through the $Ca^{2+}{}_o$-sensing receptor (CaSR), the physiologic actions of PTH, and then examine the cellular and subcellular mechanisms responsible for those actions.

The authors have reported no conflicts of interest.

PTH

During evolution, the parathyroid glands first appeared as discrete organs in amphibians (i.e., with the migration of vertebrates from an aquatic to a terrestrial existence). In mammals, PTH is produced by the parathyroid glands, although its mRNA has also been detected in the rodent hypothalamus and thymus.[1] PTH is a single chain polypeptide that comprises 84 amino acids in all investigated mammalian species, whereas chicken PTH contains 88 residues. Although fish do not have parathyroid glands, two genes encoding distinct PTH molecules that are shorter than mammalian PTH species were recently identified in zebrafish and puffer fish.[3,4] Their mRNAs are expressed in lateral line cells, which are histologically related to the mammalian vestibular apparatus and the inner ear[3]; furthermore, amino-terminal fragments of both peptides activate mammalian and fish PTH/PTHrP receptors (Fig. 1). The amino-terminal region of PTH, which is associated with most of its known biological actions, shows high homology among the different vertebrate species. The middle and carboxy-terminal regions show greater sequence variation, and these portions of the PTH molecule seem to have distinct biological properties that are probably mediated through distinct receptors.[1] However, the physiological importance of these actions needs further clarification.

Within the first 34 residues, PTH shares significant amino acid sequence conservation with PTHrP, which was initially discovered as the cause of the syndrome of humoral hypercalcemia of malignancy.[1,5] Both peptides are derived from genes that presumably evolved through an ancient gene duplication event from a common precursor and thus share similarities in their intron-exon organization. PTH and PTHrP are furthermore distantly related to the tuberoinfundibular peptide of 39 residues (TIP39),[6] and the *TIP39* gene has an organization similar to those encoding PTH and PTHrP[7] (Fig. 2).

THE PARATHYROID CELL

Regulation of PTH Synthesis and Secretion and Parathyroid Cell Proliferation

Although a large number of factors modulate parathyroid function in vitro, only a few regulators are known to be of physiological relevance in vivo.[8] The extracellular concentration of calcium ($Ca^{2+}{}_o$) is the most important determinant of the minute-to-minute secretory rate of the parathyroid gland; low $Ca^{2+}{}_o$ stimulates while high $Ca^{2+}{}_o$ inhibits PTH secretion, PTH gene expression, and parathyroid cellular proliferation, actions mediated by the CaSR. $1,25(OH)_2D_3$ inhibits expression of the PTH gene and may also directly reduce PTH secretion and parathyroid cellular proliferation. The molecular basis for vitamin D action, the VDR, a member of the superfamily of nuclear receptors, is discussed in subsequent chap-

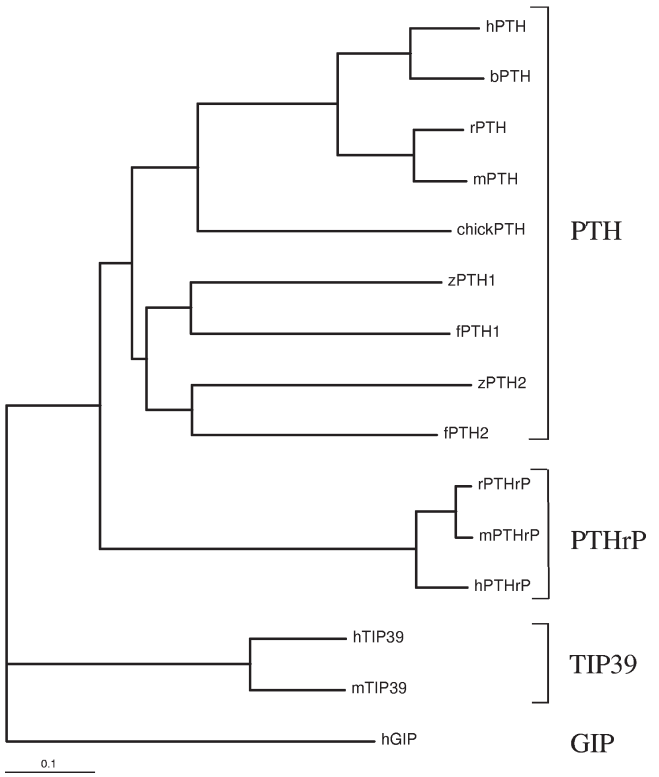

FIG. 1. Phylogenetic analysis of some precursors of PTH, PTHrP, and TIP39, and human GIP.[4]

ters. Phosphate, the most recently recognized direct modulator of parathyroid function, stimulates PTH gene expression, and, probably indirectly, PTH secretion, as well as parathyroid cellular proliferation[9] (Fig. 3). The mechanisms underlying phosphate-sensing by the parathyroid cell are not understood.

The parathyroid cell has a temporal hierarchy of responses to changes in Ca^{2+}_o, which can mount a progressively larger increase in PTH secretion in response to prolonged hypocalcemia.[8] To meet acute hypocalcemic challenges, PTH, stored in secretory vesicles, is rapidly secreted by exocytosis (e.g., over seconds to a few minutes). For the correction of prolonged hypocalcemia, parathyroid cells reduce the intracellular degradation of PTH (over minutes to an hour or so), increase PTH gene expression (over several hours to a few days), and enhance the proliferative activity of parathyroid cells (over days to weeks or longer). Many, if not all, of these processes are controlled by the G protein–coupled, CaSR—described in more detail later—that recognizes extracellular calcium ions as its principal physiological ligand (Fig. 3). This CaSR is expressed on the surface of parathyroid cells and several other cell types that are involved in regulating mineral ion homeostasis.[10]

Physiological Regulation of PTH Secretion

There is a steep inverse sigmoidal relationship between PTH levels and Ca^{2+}_o in vivo and in vitro.[8] The steepness of this curve ensures large changes in PTH for small alterations in Ca^{2+}_o and contributes importantly to the near constancy with which Ca^{2+}_o is maintained in vivo. Parathyroid cells readily detect alterations in Ca^{2+}_o of only a few percent. The mid-point or set-point of this parathyroid function curve is a key determinant of the level at which Ca^{2+}_o is "set" in vivo. The parathyroid cell responds to changes in Ca^{2+}_o within a matter of seconds, and it has sufficient stored PTH to sustain a maximal secretory response for 60–90 minutes. $1,25(OH)_2D_3$ reduces PTH secretion in vitro,[11] whereas elevations in the extracellular phosphate concentration stimulate PTH secretion.[9] These changes in PTH secretion caused by $1,25(OH)_2D_3$ and phosphate are, however, not immediate and may reflect primary actions on PTH synthesis.

Regulation of Intracellular Degradation of PTH

The pool of stored, intracellular PTH in the parathyroid cell is finite, as just noted, lasting about 1 h. The cell must therefore have mechanisms to increase hormone synthesis and release in response to more sustained hypocalcemia. One such adaptive mechanism is to reduce the intracellular degradation of the hormone, thereby increasing the net amount of intact, biologically active PTH that is available for secretion. During hypocalcemia, the bulk of the hormone that is released from the parathyroid cell is intact PTH(1-84). As the level of Ca^{2+}_o increases, a greater fraction of intracellular PTH is degraded. With overt hypercalcemia, the majority of the secreted immunoreactive PTH consists of smaller carboxy-terminal fragments. However, at least two additional forms of PTH are also secreted, including a peptide that is truncated at the amino terminus and a modified, possibly phosphorylated form of

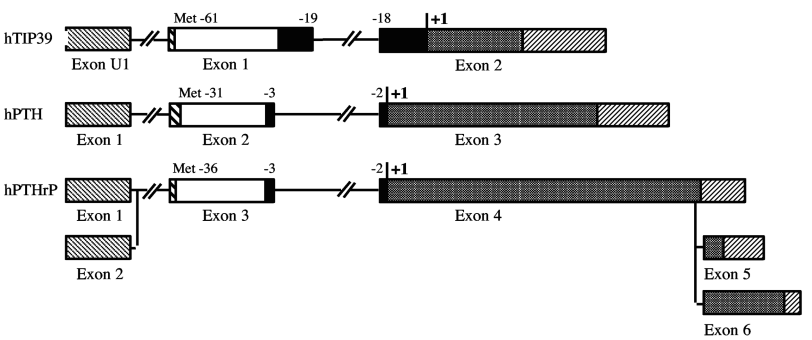

FIG. 2. Structures of the genes encoding human TIP39, human PTH, and human PTHrP. Boxed areas are exons and their names are shown underneath (because the start of exon U1 of the *TIP39* gene is unknown, the box is open on the left side), white boxes denote presequences, black boxes denote prosequences (for TIP39 presumed), gray stippled boxes denote the mature sequences; noncoding regions are shown as striped boxes. The small striped boxes preceding the white boxes denote untranslated exonic sequences (4 bp for TIP39; 5 bp for PTH; 22 bp for PTHrP). The positions of the initiator methionine based on the secreted peptide are noted above the graphs; the positions where pro-sequences are interrupted by an intron are noted above the graph. +1 denotes the relative position of the beginning of the secreted peptide.[7]

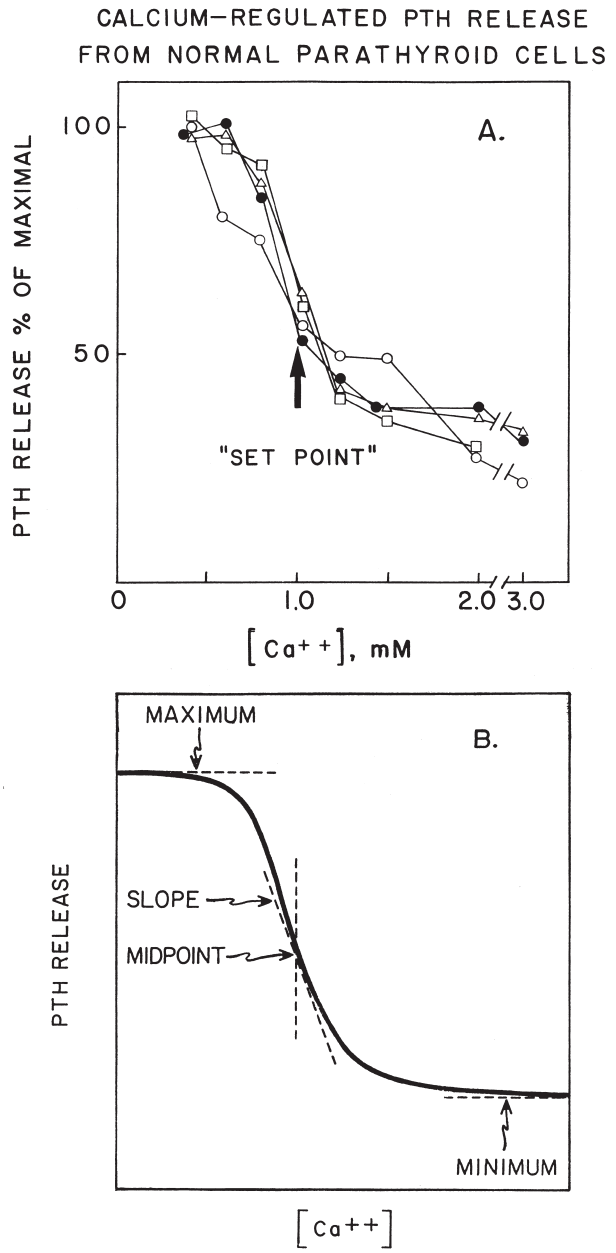

FIG. 3. (A) Relationship between PTH secretion and extracellular calcium in normal human parathyroid cells. Dispersed parathyroid cells were incubated with the indicated levels of calcium and PTH was determined by radioimmunoassay. (Reproduced with permission from Brown EM 1980 Set-point for calcium: Its role in normal and abnormal parathyroid secretion. In: Cohn DV, Talmage RV, Matthews JL (eds.) Hormonal Control of Calcium Metabolism, Proceedings of the Seventh International Conference on Calcium Regulating Hormones, September 5–9, 1980, International Congress series No. 511, Excerpta Medica, Amsterdam, pp. 35–43. (B) The four parameters describing the inverse sigmoidal relationship between the extracellular calcium concentration and PTH release in vivo and in vitro: A, maximal secretory rate; B, slope of the curve at the midpoint; C, midpoint or set-point of the curve (the level of calcium producing one-half of the maximal decrease in secretory rate; D, minimal secretory rate.[61]

PTH(1-84).[12] The amino-terminally truncated form of PTH(1-84) seems to be identical to PTH(7-84),[12] which was previously shown to bind to a unique receptor, reduce in vitro the formation of osteoclast-like cells, and has hypocalcemic properties when tested in vivo.[13]

Physiological Control of PTH Gene Expression

The second adaptive mechanism of the parathyroid cell to sustained reductions in Ca^{2+}_o is to increase the cellular levels of PTH mRNA, which takes several hours. Reductions in Ca^{2+}_o increase, whereas elevations reduce, the cellular levels of PTH mRNA by affecting the transcription rate of the PTH gene as well as through additional, post-transcriptional mechanisms.[2] Available data suggest that phosphate ions also directly regulate PTH gene expression. Hypo- and hyperphosphatemia in the rat lower and raise, respectively, the levels of mRNA for PTH through a mechanism of action that is independent of changes in Ca^{2+}_o or $1,25(OH)_2D_3$.[2] This action of an elevated extracellular phosphate concentration could potentially contribute importantly to the secondary hyperparathyroidism frequently encountered in states with a chronically high serum phosphate, such as the secondary hyperparathyroidism in end-stage renal failure. It will be of interest to determine whether phosphate-sensing involves a receptor-mediated mechanism similar to that through which Ca^{2+}_o regulates parathyroid and kidney function.

Metabolites of vitamin D, principally $1,25(OH)_2D_3$, play an important role in the long-term regulation of parathyroid function and may act at several levels, including the control of PTH secretion,[11] as noted before, control of PTH, CaSR, and VDR expression,[8,14] as well as the regulation of parathyroid cellular proliferation.[2] By far the most important metabolite of vitamin D modulating parathyroid function is $1,25(OH)_2D_3$, which acts principally through an intracellular receptor that functions as a nuclear transcription factor, often in concert with other such transcription factors (i.e., those for retinoic acid or glucocorticoids). $1,25(OH)_2D_3$ reduces the levels of the mRNA encoding PTH through an action mediated by DNA sequences upstream from the PTH gene. $1,25(OH)_2D_3$-induced upregulation of the level of VDR expression in parathyroid could act as a feedforward mechanism to potentiate its own inhibitory action(s) on parathyroid function.[2] High Ca^{2+}_o and $1,25(OH)_2D_3$ coordinately increase the mRNA for the VDR.[8] Some of the "noncalcemic" analogs of $1,25(OH)_2D_3$ (e.g., 22-oxacalcitriol, calcipotriol, and 19-nor-1,25-dihydroxyvitamin D_2) inhibit PTH secretion while producing relatively little stimulation of intestinal calcium absorption and of bone resorption, the biological actions that underlie the hypercalcemic effects of $1,25(OH)_2D_3$.[15] Therefore, these synthetic vitamin D analogs may represent attractive candidates for treating the hyperparathyroidism of chronic renal insufficiency, because hypercalcemia resulting from the gastrointestinal and skeletal actions of $1,25(OH)_2D_3$ often becomes a factor limiting the treatment of such patients. In addition to the kidney, the parathyroid cell expresses the 25-hydroxyvitamin D_3 1-hydroxylase that forms $1,25(OH)_2D_3$ from 25-hydroxyvitamin D_3. Therefore, raising the level of 25-hydroxyvitamin D_3 (e.g., by administering vitamin D_3) may raise the level of $1,25(OH)_2D_3$ within the parathyroid cell in patients with end-stage renal failure and represent another means of lowering PTH levels in this setting.[16]

Physiological Regulation of Parathyroid Cellular Proliferation

The final adaptive mechanism contributing to changes in the overall level of parathyroid gland secretory activity is to adjust the rate of parathyroid cellular proliferation. Under normal conditions, there is little or no proliferative activity of parathyroid cells. The parathyroid glands, however, can enlarge greatly during states of chronic hypocalcemia, particularly in the setting of renal failure [probably because of a combination of hypocalcemia, hyperphosphatemia, and low levels of

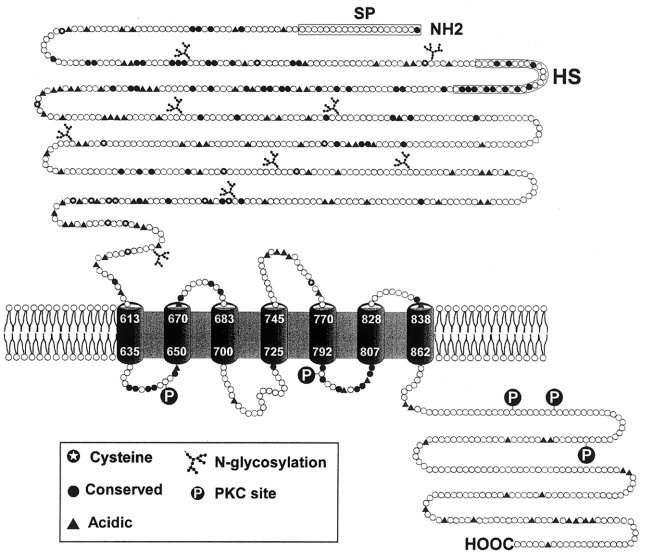

FIG. 4. Predicted structure of the human CaR. SP, signal peptide; HS, hydrophobic segment.[62]

$1,25(OH)_2D_3$ in the latter condition].[8] This enlargement cannot be accounted for solely by cellular hypertrophy, although the latter does contribute to the overall increase in glandular mass. The ability of calcium administration to prevent parathyroid hyperplasia in mice with deleted vitamin D receptors shows the importance of calcium in regulating parathyroid cell number.[17] Calcium acts to prevent hyperplasia through activation of the CaSR, because mutational inactivation of the CaSR in humans and mice leads to parathyroid hyperplasia at birth and because calcimimetic compounds that activate the CaSR can prevent parathyroid hyperplasia in experimental uremia.[18]

Molecular Basis for Ca^{2+}_o Sensing by the Parathyroid Cell

Recent studies have elucidated the mechanism by which Ca^{2+}_o regulates several of the aspects of parathyroid function described above. For many years, it was unclear how an ion could regulate cellular function, other than through actions on ion channels, membrane potential, and the like. The realization that the calcium ion could control the function of parathyroid and other cells through a G protein–coupled receptor (GPCR) contributed to the current awareness that a much wider range of agents affect cellular function through GPCRs than previously recognized (e.g., lipids, bile acids, citric acid cycle intermediates).

The CaSR is the molecular mechanism underlying the sensing of Ca^{2+}_o by the parathyroid chief cells as well as other cells involved in calcium homeostasis, particularly the kidney.[19] In effect, the CaSR acts as the body's thermostat for calcium or "calciostat." It is capable of detecting perturbations in the serum ionized calcium concentration of only a few percent and responding with the alterations in parathyroid function described above as well as changes in renal function that are designed to normalize Ca^{2+}_o. The CaSR has three structural domains: a large extracellular "sensing" domain (ECD), the seven membrane-spanning "serpentine" motif characteristic of the GPCRs, and a long intracellular, carboxyterminal (C)-tail (Fig. 4).[19] The ECD of the CaSR is heavily glycosylated, which is important for its efficient cell surface expression. The biologically active form of the receptor is a dimer, linked together by two disulfide bonds (at cys129 and cys131) between the ECDs of two monomers. The CaSR's ECD has important determinants for binding extracellular calcium ions, although the locations of these sites are currently unknown, and some binding of calcium to the transmembrane domains may occur as well. After the binding of calcium to the ECD, the initiation of CaSR signaling involves the binding of G proteins to the receptor's intracellular loops—especially the second and third loops—and the proximal portion of the C-tail.[20] In addition to activating phospholipases A_2, C, and D, the CaSR also activates various mitogen-activated protein kinases (MAPKs) and inhibits adenylate cyclase.[18]

While Ca^{2+}_o is undoubtedly the CaSR's principal physiological ligand in vivo, the receptor is activated by a number of other ligands, at least two of which—magnesium and certain amino acids[21]—are likely to be physiologically relevant. Although magnesium is ~2-fold less potent than calcium in its actions on the CaSR, and the level of Mg^{2+}_o is lower than that of Ca^{2+}_o, persons with inactivating or activating mutations of the receptor tend to have increases or decreases in their serum magnesium concentrations, respectively. These alterations in serum magnesium encompass changes within the normal range to frank hyper- or hypomagnesemia. Thus, it is likely that the CaSR contributes to "setting" the normal level of extracellular magnesium.

More recent studies have shown that certain amino acids, especially aromatic amino acids, allosterically activate the CaSR,[21] effectively sensitizing the receptor to any given level of Ca^{2+}_o. It is possible, therefore, that the CaSR serves a more generalized role as a "nutrient" receptor, recognizing not only divalent cations but also amino acids. For instance, both calcium and aromatic amino acids increase gastrin release and acid production in the stomach—actions that are likely mediated by the CaSR.[19] There are additional circumstances in which calcium and protein metabolism seem to be linked in ways that could be mediated by the CaSR. A high protein intake promotes hypercalciuria, an action traditionally ascribed to the acid load generated by metabolism of the protein; however, stimulation of renal CaSRs by high circulating levels of amino acids could also contribute to the hypercalciuria. Furthermore, a low protein intake in normal subjects, as well as in patients with renal impairment, is associated with elevated levels of PTH.[22] This association could be mediated, in part, by the parathyroid glands sensing a reduction in "nutrient" availability (e.g., the sum of divalent cation and amino acids and responding with enhanced PTH secretion).

In addition to these endogenous ligands, allosteric activators of the CaSR, so-called "calcimimetics," have been developed, as have CaSR antagonists, termed "calcilytics."[23] Calcimimetics are currently in clinical trials for the treatment of primary hyperparathyroidism and have been approved for use in controlling secondary hyperparathyroidism in patients with chronic renal insufficiency undergoing dialysis treatment. They seem to provide an effective treatment in the latter setting and restore calcium to or toward normal in primary hyperparathyroidism. Calcilytics provide a means of stimulating endogenous PTH secretion by "tricking" the parathyroid glands into sensing hypocalcemia. They may provide an alternative to the injection of PTH and its analogs as an anabolic treatment of osteoporosis.[23]

Studies of mice homozygous for targeted disruption of the *CaSR* gene[24] and of patients with activating or inactivating mutations of the receptor[14] have firmly established the role of the CaSR in regulating several of the aspects of parathyroid function that were discussed above. With complete knockout of the CaSR, there is markedly abnormal Ca^{2+}_o-regulated PTH release, which is poorly, if at all, regulated by extracellular calcium. There is also marked parathyroid hyperplasia in this

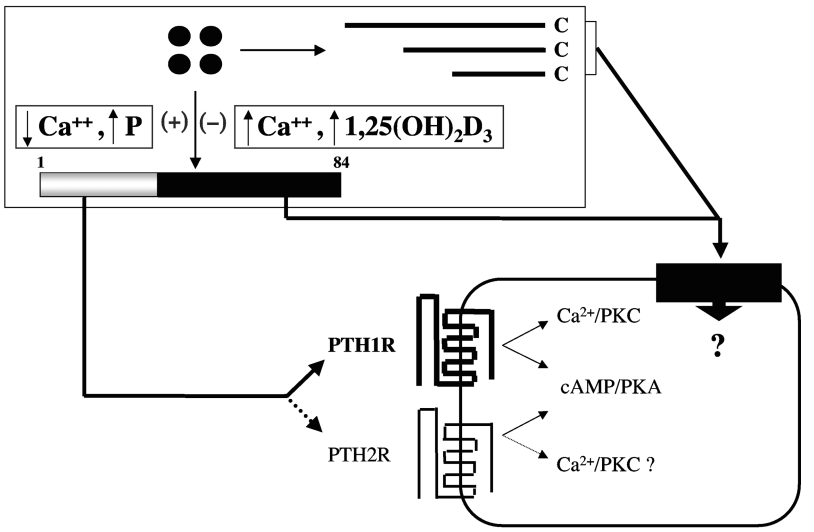

FIG. 5. PTH production and activation of different receptors. Intact PTH and different fragments are secreted from the parathyroid glands. Low ionized calcium and elevated phosphate increase PTH synthesis and secretion, whereas increased ionized calcium and $1,25(OH)_2D$ lead to a decrease; note that the regulatory actions of calcium are mediated through the calcium-sensing receptor. Different receptors interact with the amino- or carboxy-terminal portion of intact PTH. Through its amino-terminal portion, PTH activates the PTH/PTHrP receptor (PTH1R), a G protein–coupled receptor that mediates its actions through at least two different signaling pathways, cAMP/KPA and Ca^{2+}/PKC. The closely related PTH2 receptor (PTH2R) is most likely the primary receptor for the tuberoinfundibular peptide of 39 residues (TIP39); however, at least the human PTH2R is also activated by amino-terminal PTH. Another receptor, which has not yet been cloned, interacts only with the carboxy-terminal portion of PTH.

setting, establishing that the CaSR, directly or indirectly, regulates parathyroid cellular proliferation. Available data also suggest that the receptor mediates the inhibitory effect of high Ca^{2+}_o on PTH gene expression. Additional studies will be needed to determine the receptor's role in the regulation of other aspects of parathyroid function by Ca^{2+}_o, such as intracellular degradation of PTH.

In addition to being expressed in the parathyroid chief cells, the CaSR is also expressed by the calcitonin-secreting C cells of the thyroid gland, where it mediates the stimulation of CT secretion by Ca^{2+}_o.[19] Calcitonin probably does not participate to a meaningful extent in maintaining calcium homeostasis in humans, but its hypocalcemic actions—exerted through inhibition of bone resorption and stimulation of renal calcium excretion—are robust in other species, particularly rodents. It serves, therefore, as part of a homeostatic loop in which hypercalcemia stimulates calcitonin secretion, whose hypocalcemic actions, when combined with the homeostatic action of lowering PTH levels, foster a return to normocalcemia.

Molecular Defects in the CaSR

Soon after the cloning of the CaSR, families with familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism were found in most cases to harbor inactivating mutations in the *CaSR* gene. Subsequently, activating mutations in the receptor were identified as the cause of a form of hypoparathyroidism called autosomal dominant hypoparathyroidism (ADH or ADHP). In addition to providing the molecular basis for these inherited conditions of calcium metabolism, the clinical and biochemical features of these disorders has provided important insights into the CaSR's role in calcium and water metabolism as well as into the structure and function of the receptor.[14]

In familial hypocalciutic hypercalcemia (FHH), there is PTH-dependent hypercalcemia accompanied by PTH-independent hypocalciuria, firmly establishing the role of the CaSR in Ca^{2+}_o-regulated PTH release as well as in renal handling of calcium. Reduced CaSR activity, likely in the cortical thick ascending limb (CTAL), leads to constitutive reabsorption of calcium with a conspicuous lack of the usual calciuric action of hypercalcemia mediated by the receptor. These individuals also concentrate their urine more effectively than a patient with primary hyperparathyroidism and a comparable degree of hypercalcemia,[25] supporting the CaSR's role in inhibiting the renal concentrating mechanism. Finally, the prominent lack of symptoms in FHH supports a role for the CaSR in mediating some of the symptomatology of hypercalcemia.

In contrast to FHH, neonatal severe hyperparathyroidism (NSHPT) is a severe disorder caused, in its worst cases, by the presence of homozygous or compound heterozygous mutations in the CaSR.[14] As in mice homozygous for knockout of the CaSR, there is marked, PTH-dependent hypercalcemia with hyperparathyroid bone disease. The disorder can be fatal without urgent parathyroidectomy. Thus, the total absence of normally functioning CaSRs can be incompatible with life. The clinical and biochemical manifestations of ADH are the converse of FHH, and the former might also be termed familial hypercalciuric hypocalcemia.[26] The presence of CaSRs with inappropriately high activity at any given level of Ca^{2+}_o inhibits PTH secretion and promotes an excessive degree of urinary calcium excretion for a given level of Ca^{2+}_o. Of interest, as the level of serum calcium concentration is increased in these patients by treatment with $1,25(OH)D_3$ and calcium supplementation, they can develop symptoms suggesting hypercalcemia, polydipsia, and polyuria, even while still hypocalcemic, again supporting the CaSR as a mediator of symptoms of hypercalcemia as well as the link between calcium and water metabolism noted above.

PTH ACTION

Receptors for PTH

PTH-dependent regulation of mineral ion homeostasis is largely mediated through the PTH/PTHrP receptor, which is coupled to adenylate cyclase through $G_s\alpha$ and to phospholipase C through the G_q family of signaling proteins.[1,27] (see Fig. 5). While most PTH/PTHrP receptor–dependent actions involve activation of adenylyl cyclase, some actions seem to require phospholipase C–mediated events. These dual signaling properties are particularly relevant, because the PTH/PTHrP receptor was recently shown to interact in vitro, through a PDZ domain, with $Na^{(+)}/H^{(+)}$ exchange regulatory factors sodium-hydrogen exchanger regulatory factors (NHERF) 1 and NHERF2. In the presence of NHERF2 (but probably also NHERF1), the activated PTH/PTHrP receptor preferentially activated phospholipase C and inhibited adenylyl cyclase through stimulation of inhibitory G proteins ($G_{i/o}$ proteins).[28]

NHERF-dependent changes in PTH/PTHrP receptor signaling may thus account for some of different tissue- and cell-specific actions induced by PTH or PTHrP.

The PTH/PTHrP receptor belongs to a distinct family of G protein–coupled receptors and mediates with similar or indistinguishable efficacy biological actions of both PTH and PTHrP.[1,27] The PTH/PTHrP receptor is most abundantly expressed in the target tissues for PTH's actions (i.e., kidney and bone), but it is also found in a large variety of other fetal and adult tissues, and at particularly high concentrations in growth plate chondrocytes.[1,27] In tissues other than kidney and bone, the PTH/PTHrP receptor most likely mediates the para-/autocrine actions of PTHrP rather than the endocrine actions of PTH. Of considerable importance is the receptor's role in cartilage and bone development, because it mediates in this tissue the PTHrP-dependent regulation of chondrocyte proliferation and differentiation; thus, it has a major role in bone development and growth.[29]

The PTH/PTHrP receptor seems to be the most important receptor mediating the actions of PTH and PTHrP. There is considerable pharmacologic evidence, however, for the existence of other receptors that are activated by either PTH and/or PTHrP, including a receptor/binding protein that interacts with the carboxy-terminal portion of PTH and may be involved in mediating the hypocalcemic actions of this portion of the molecule.[13] However, most of these putative receptors have not yet been cloned and their biological functions, some of which may be unrelated to the control of calcium and phosphorus homeostasis, remain poorly characterized. Only cDNA encoding the PTH2 receptor has been isolated thus far.[1,27,30] Only the human PTH2 receptor, but not the homolog of this receptor from other species, is activated by PTH; PTHrP does not activate any of the different PTH2 receptor species unless residues 5 and 23 are replaced with the corresponding PTH-specific amino acids.[1,27] However, the natural ligand for the PTH2 receptor seems to be TIP39, a recently identified hypothalamic peptide.[6] Expression of the PTH2 receptor is restricted to relatively few tissues (i.e., placenta, pancreas, blood vessels, testis, and brain), and although most biological function(s) mediated through this receptor remain to be determined, it may have a role in the regulation of renal blood flow.[30,31]

Actions of PTH on Bone

PTH has complex and only partially understood actions on bone that require the presence of and often direct contact between several different specialized cell types, including osteoblasts, bone marrow stromal cells, hematopoietic precursors of osteoclasts, and mature osteoclasts.[32] Administration of PTH leads to the release of calcium from a rapidly turning-over pool of calcium near the surface of bone; after several hours, calcium is also released from an additional pool that turns over more slowly.[33] Chronic administration of PTH (or increased secretion of PTH associated with primary hyperparathyroidism) leads to an increase in osteoclast cell number and activity.[34] The release of calcium is accompanied by the release of phosphate and matrix components, such as degradation products of collagen. Paradoxically, particularly when given intermittently, PTH administration leads to the formation of increased amounts of trabecular bone[35,36]; these anabolic actions of PTH are currently being explored for the prevention and treatment of osteoporosis, either alone or in combination with a bisphosphonate.[37–39]

The osteoblast and its precursor, the marrow stromal cell, have central roles in directing both the catabolic (bone resorption) and anabolic (bone formation) actions of PTH (Fig. 6). Only a subset of stromal cells and osteoblasts synthesize

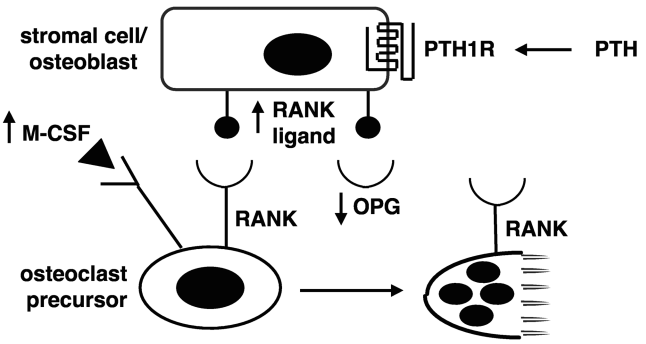

FIG. 6. PTH actions on bone. The PTH/PTHrP receptor (PTH1R) is expressed on stromal cells/osteoblasts. On receptor activation by PTH, expression of M-CSF and RANKL are increased, which enhances the formation of osteoclasts from precursors and the activity of already existing mature osteoclasts. In response to PTH, expression of OPG, a decoy receptor for RANKL, is decreased, thus reducing the activity of existing osteoclasts and the formation of mature osteoclasts from precursor cells.

mRNA encoding the PTH/PTHrP receptor.[1] Although cell lines capable of differentiating into osteoclasts have been shown to have PTH/PTHrP receptors, these receptors are not needed or sufficient for the stimulation of osteoclastic development by PTH. Elegant studies of co-cultures of osteoblasts/stromal cells and osteoclast precursors had shown that PTH affects osteoclast maturation and functions only indirectly through its actions on cells of the osteoblast lineage, which express abundant amounts of the PTH/PTHrP receptor.[32] A key osteoblastic protein that activates osteoclast development and activity of mature osteoclasts is RANKL (also termed osteoclast-differentiating factor [ODF], TRANCE, or osteoprotegerin ligand), a member of the TNF family of proteins, which is anchored by a single hydrophobic membrane-spanning domain to the cell surface of osteoblasts.[32] On interaction with RANK, a member of the TNF receptor family, expressed on pre-osteoclasts, these precursors differentiate into mature osteoclasts, if macrophage-colony stimulating factor (M-CSF) is also present.[32] RANKL also increases the bone-resorbing properties of mature osteoclasts. PTH stimulates the expression of RANKL on the cell surface of osteoblasts, and the same response is stimulated by other molecules [i.e., interleukin 11 (IL-11), prostaglandin E_2, and $1,25(OH)_2D$] that were previously noted to stimulate the formation of osteoclasts. PTH also stimulates the synthesis of M-CSF.[32]

The interactions of osteoblastic RANKL and its osteoclastic receptor RANK are further controlled by a secreted protein, osteoprotegerin (OPG), a soluble "decoy" receptor with homology to RANK and other members of the TNF receptor family. The effects of overexpression of OPG in transgenic mice and the ablation of the *OPG* gene both suggest that this binding protein importantly modulates the communication between osteoblasts and osteoclasts.[32] PTH inhibits the expression of OPG in osteoblast cells. Thus, by increasing M-CSF and RANKL and inhibiting OPG expressed locally by cells of the osteoblast lineage, PTH stimulates osteoclastogenesis and the activity of mature osteoclasts.

The mechanisms whereby PTH increases bone formation are complicated and less well understood (see Fig. 4). PTH increases the number of osteoblasts by increasing the number of osteoprogenitors cells[40] as a result of a reduction in apoptosis of pre-osteoblasts and osteoblasts,[41] by increasing osteoblast proliferation, and perhaps by converting inactive bone-lining cells to active osteoblasts.[42] On the other hand, when added to cells in culture, PTH stops pre-osteoblastic cells from becoming mature osteoblasts. It also changes the activity of mature

osteoblasts. In cell culture systems, PTH inhibits the production of collagen and other matrix proteins, perhaps partly by steering the key osteoblast transcription factor, RUNX2 (also called CBFA-1), to proteosome-mediated destruction.[42] The prominent action of PTH in vivo to increase bone formation may result from the production by osteoblasts of growth factors such as IGF-1 and fibroblast growth factor (FGF)-2, as well as from the release of growth factors from matrix after PTH-induced osteoclast action.[43]

With complicated actions both on bone formation and bone resorption, it is perhaps not surprising that the net effects of PTH on bone can be either anabolic (net increase in bone mass) or catabolic (net decrease in bone mass). Depending on the dose of PTH, the mode of administration (intermittent versus continuous), the animal species, and the specific site (trabecular bone versus cortical bone), PTH can be either anabolic or catabolic.

Actions of PTH in Kidney

In the kidney, PTH has three major biological functions that are essential for the regulation of mineral ion homeostasis: stimulating the reabsorption of calcium, inhibiting the reabsorption of phosphate, and enhancing the synthesis of $1,25(OH)_2D_3$. Each of these actions of PTH contributes to the maintenance of blood calcium, and to a lesser extent, phosphate concentrations within narrow limits.

Phosphate is normally reabsorbed from the glomerular filtrate both in the proximal and distal tubules, and reabsorption is inhibited by PTH at both these sites.[44] Best studied is its effect on proximal tubular cells where phosphate is transported into the cell against an electrochemical gradient. To accomplish this task, an ATP-dependent sodium pump, Na^+/K^+ ATPase, drives sodium from the cell. Because of the concentration gradient for sodium established by this pump and through the actions of two membrane-anchored co-transporters, NPT-2a and NPT-2c, sodium re-enters the cell along with phosphate.[44–46] PTH blocks this sodium-dependent phosphate co-transport by reducing the amount of the Npt2a and possibly Npt2c protein on the cell surface, primarily by increasing its internalization and subsequent lysosomal degradation, but also by decreasing its synthesis. PTH is only one of several determinants of Npt2a and Npt2c expression, as dietary phosphate restriction leads, independent of changes in blood concentrations of PTH, to a markedly enhanced renal phosphate reabsorption, and thus a virtual elimination of urinary phosphate losses.[44–46] Furthermore, FGF-23 is a recently identified, key regulator of phosphate homeostasis, which also has direct actions on Npt2a and Npt2c expression.[46,47] The complete lack of Npt2a expression through ablation of its gene leads to severe renal phosphate wasting and nephrocalcinosis.[47] Although these abnormalities are similar to those observed in hereditary hypophosphatemic rickets with hypercalciuria (HHRH), mutations in *Npt2a* were excluded in this disorder; instead homozygous or compound heterozygous mutations were identified in NPT2c.[49,50] Interestingly, heterozygous *NPT2a* mutations were recently identified in two patients with nephrolithiasis and osteoporosis associated with hypophosphatemia caused by impaired renal tubular reabsorption.[51] These seem to have a dominant negative effect, although the in vitro findings leading to this conclusion are controversial.[52]

In the distal tubule, PTH also inhibits phosphate reabsorption; the transporter(s) that are involved in this process have not yet been identified. Teleologically, the PTH-stimulated phosphaturia can be viewed as a way of handling the release of phosphate from bone that accompanies the PTH-stimulated release of calcium from bone. Furthermore, because of the quantitative dominance of the phosphaturia, blood phosphate falls in response to PTH. This hypophosphatemia reinforces the effect of PTH on bone, because low levels of blood phosphate stimulate bone resorption.

Most calcium reabsorption occurs in the proximal tubule, but only the calcium reabsorption in the distal nephron is PTH dependent.[44] In the CTAL, reabsorption of NaCl, Ca^{2+}, and Mg^{2+} takes place largely through a paracellular pathway and is driven by the lumen positive transepithelial potential in this nephron segment. PTH stimulates calcium reabsorption in the CTAL by increasing the value of this potential through a cAMP-dependent mechanism. PTH also is thought to enhance the transcellular reabsorption of calcium in the CTAL.[44] In the distal convoluted tubule (DCT), reabsorption of calcium and magnesium take place through the transcellular route—a process that is augmented by PTH. Calcium initially enters the cells through a recently cloned, calcium-permeable channel in the luminal membrane called TRPV5[53] and exits the basolateral membrane through the Na^+/Ca^{2+} exchanger and/or Ca^{2+}-ATPase.

Although the kidneys reabsorb calcium more efficiently when stimulated by PTH, the absolute amount of calcium in the urine usually increases when the circulating concentrations of PTH are chronically elevated to levels sufficient to produce hypercalcemia, as in patients with primary hyperparathyroidism. However, this increase in urinary calcium excretion is caused by the substantial increase in the filtered load of calcium, which is caused by the hypercalcemia that results from increased bone resorption and increased intestinal absorption of calcium rather than by impaired renal tubular reabsorption of calcium.

PTH also activates the mitochondrial 25-hydroxyvitamin D_3-1-α-hydroxylase in proximal tubular cells; this leads to an elevation of the blood $1,25(OH)_2D_3$ concentration, which, in turn, is a potent inducer of intestinal calcium absorption (as well as of bone resorption).[44] This effect of PTH is not immediate, because the stimulation of $1,25(OH)_2D_3$ synthesis occurs over several hours and requires the synthesis of new mRNA and protein. Along with its action on the 1-α-hydroxylase, PTH decreases the activity of the renal 25-hydroxyvitamin D_3-24-hydroxylase, thus enhancing the effect on $1,25(OH)_2D_3$ synthesis. Other factors, particularly low blood phosphate concentration, also markedly increase the synthesis of this biologically active vitamin D metabolite, whereas hypercalcemia, as would be generated by sustained increases in PTH, directly suppresses, independent of blood levels of PTH or phosphate, the 1-α-hydroxylase activity, and thus limits in a homeostatic manner the production of $1,25(OH)_2D_3$.[44]

Because of its effectiveness in raising the blood calcium concentration, $1,25(OH)_2D_3$ is widely used, along with oral calcium supplementation, in the treatment of hypoparathyroidism and pseudohypoparathyroidism.[54,55] However, because $1,25(OH)_2D_3$ cannot mimic the renal, calcium-sparing effects of PTH, urinary calcium excretion can rise quickly as serum calcium approaches the normal range, particularly when the underlying hypoparathyroidism is caused by activating mutations in the calcium-sensing receptor, as in autosomal dominant hypocalcemia with hypercalciuria.[14] In both groups of patients, but particularly the latter, the blood calcium is best kept at or below the lower limit of the normal range, with periodic monitoring of 24-h urinary calcium excretion, to avoid the long-term consequences of hypercalciuria.

Direct, CaSR-Mediated Modulation of Renal Actions of PTH

In addition to being expressed by parathyroid chief cells and thyroidal C cells, the CaSR is also expressed in numerous nephron segments, where it modulates several of the renal actions of PTH. In this way, the receptors for PTH and extracellular calcium can exert mutually antagonistic actions on the kidney that enable integrated control of tubular function not only by PTH per se but also by a key regulator of PTH secretion (e.g., Ca^{2+}_o, acting through the CaSR). Thus, in addition to its long-recognized role in regulating PTH secretion, extracellular calcium also modulates PTH action. Calcium exerts numerous actions on the kidney, several of which are relevant to the physiology and pathophysiology of mineral ion metabolism.[14] For example, high Ca^{2+}_o inhibits the 1-hydroxylation of 25-hydroxyvitamin D_3, reduces renin secretion, promotes hypercalciuria, and reduces urinary concentrating ability. Recent data have implicated a mediatory role of the CaSR in several of these, as noted below.

Prominent sites of CaSR expression in the kidney are on the apical surface of the proximal tubular cells, the basolateral surface of the medullary (MTAL) and CTALs of Henle's loop as well as the macula densa.[56] It is present predominantly on the basolateral membrane of the DCT. In the most distal nephron, it resides on the apical surface of the inner medullary collecting duct (IMCD), where vasopressin increases renal tubular reabsorption of water during dehydration by promoting insertion of aquaporin-2–containing endosomes into the same apical membrane.

In the proximal tubule, available data indicate that the CaSR blunts the phosphaturic action of PTH, probably by inhibiting PTH-stimulated cAMP accumulation. It has not yet been determined whether the CaSR in the proximal tubule also mediates the inhibitory action of hypercalcemia on the 1-hydroxylation of 25-hydroxyvitamin D_3, which occurs not only indirectly through CaSR-induced reduction in PTH secretion, but also through a direct suppressive action of hypercalcemia on the proximal tubular cells.

In the MTAL, the CaSR inhibits NaCl reabsorption, thereby impairing generation of the hypertonic interstitium required for vasopressin to maximally stimulate water reabsorption in the IMCD. As will be discussed below, the CaSR also inhibits the action of vasopressin in the IMCD,[57] further limiting the capacity of the kidney to concentrate the urine. These two actions of the CaSR on the renal concentrating mechanism very likely account for the known inhibitory effect of hypercalcemia on urinary concentrating ability.[58]

The CaSR inhibits calcium reabsorption in both the CTAL and DCT, including that stimulated by PTH. Thus, in addition to decreasing renal calcium reabsorption by CaSR-mediated inhibition of PTH secretion, the CaSR also blocks the action of PTH on these nephron segments to further reduce renal calcium and magnesium reabsorption by a direct renal action of Ca^{2+}_o. In both nephron segments, the CaSR likely acts by reducing PTH-stimulated cAMP accumulation, but may also act through additional intracellular signaling pathways.

In the IMCD, available data strongly implicate the CaSR in modulating vasopressin-stimulated water reabsorption in a physiologically important manner.[57,58] Consistent with the CaSR's presence on the apical but not the basolateral surface of the IMCD, perfusion of the luminal (but not the basolateral side) of isolated IMCD segments with elevated levels of Ca^{2+}_o inhibits vasopressin-stimulated water flow by about 40%. Vasopressin stimulates water reabsorption in this nephron segment by promoting aquaporin-2–mediated reabsorption of water into the hypertonic medullary interstitium. Because vasopressin exerts its effect on aquaporin-2 trafficking by activating adenylate cyclase, the CaSR could inhibit this process, at least in part, by inhibiting vasopressin-stimulated cAMP accumulation, similar to its actions on PTH-stimulated cAMP accumulation noted above.[58] By inhibiting vasopressin-stimulated water flow, the CaSR would, in effect, set an upper limit to the level of Ca^{2+}_o that could be reached within the distal nephron, which could potentially reduce the risk of renal stone formation in hypercalciuric states, such as primary hyperparathyroidism.

Molecular Defects in the PTH/PTHrP Receptor

The endocrine actions of PTH, and the autocrine/paracrine actions of PTHrP, are mediated through the PTH/PTHrP receptor.[1] A single G protein–coupled receptor is thus essential for the biological roles of two distinct ligands, which are important for regulation of calcium homeostasis and for the regulation of chondrocyte proliferation and differentiation, respectively. As with the CaSR, genetic deletion of the PTH/PTH receptor gene in mice and the identification of naturally occurring mutations in this receptor in humans have shed considerable light on the receptor's physiological roles in vivo.

The ablation of one allele encoding the PTH/PTHrP receptor gene in mice revealed no discernible abnormality, whereas the ablation of both alleles resulted, depending on the mouse strain, in fetal death during mid- or late gestation and severe skeletal abnormalities.[29] Based on the functional properties of the PTH/PTHrP receptor and based on the findings in gene-ablated mice, it seemed likely that receptor mutations in humans would most likely affect mineral ion homeostasis and bone development.

Mutations in the PTH/PTHrP receptor were initially suspected as a cause of pseudohypoparathyroidism type Ib (PHP-Ib), in which patients exhibit PTH-resistant hypocalcemia and hyperphosphatemia.[54,55] However, these patients lack discernible growth plate abnormalities, indicating that the actions of PTHrP are appropriately mediated. It was therefore not surprising, at least in retrospect, that PTH/PTHrP receptor mutations could not be identified in PHP-Ib patients. The autosomal dominant form of PHP-Ib (AD-PHP-Ib) was subsequently mapped to the *GNAS* locus and was shown to be paternally imprinted.[54,55] Furthermore, patients affected by PHP-Ib revealed changes in the methylation pattern at one or several *GNAS* exons and their promoters.[59] Recently, several different deletions either up-stream of or within the *GNAS* locus were identified.[54,55] It remains uncertain, however, how these deletions affect methylation and lead to PTH resistance in the proximal renal tubules.

PTH/PTHrP receptor mutations have been identified in three rare genetic disorders: Jansen metaphyseal chondrodysplasia, Blomstrand lethal chondrodysplasia, and Eiken skeletal dysplasia. Activating mutations that lead to ligand-independent accumulation of cAMP were identified as the cause of the autosomal dominant Jansen disease, which is characterized by short-limbed dwarfism, severe hypercalcemia, and hypophosphatemia, despite normal or undetectable levels of PTH and PTHrP in the circulation; a milder form of this disease was found to be caused by a PTH/PTHrP receptor mutation that leads to less pronounced ligand-independent cAMP formation.[55] Inactivating PTH/PTHrP receptor mutations (homozygous or compound heterozygous) were identified in patients with Blomstrand disease, who are typically born prematurely and die at birth or shortly thereafter. These patients present with advanced bone maturation, accelerated chondrocyte differentiation, and, most likely, severe abnormalities in mineral ion homeostasis.[55] Homozygous PTH/PTHrP receptor muta-

tions were furthermore identified in Eiken skeletal dysplasia, a nonlethal disorder affecting the skeleton.[60]

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Chapter 16. Parathyroid Hormone–Related Protein

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INTRODUCTION

In 1941, Fuller Albright proposed that tumors associated with hypercalcemia might produce a PTH-like factor. Forty years later, it was recognized that the factor in question interacts with the PTH receptor in the kidney, an observation that provided the strategy that was used to isolate and clone the PTH-related protein (PTHrP) in the mid-1980s.[1–3]

We now know that the *PTH* and *PTHrP* genes arose by duplication and are members of a small gene family.[1–5] What remains from this common heritage is a similar organization of the two genes and a stretch of homologous sequence at the N terminus of each mature peptide. Otherwise, the genes have evolved separately. The *PTH* gene has a simple structure, and its product is a classical systemic peptide hormone. The *PTHrP* gene has a complex structure, is widely expressed in normal tissues, and its product(s) seems to function principally in an autocrine or paracrine fashion.[4,5] A fundamental aspect of the divergence of these two genes has been the development of very different mechanisms of control. The key regulatory step in PTH physiology is calcium-gated PTHrP secretion. In contrast, PTHrP seems to be a constitutive secretory product, such that the key control point in PTHrP production/secretion is at the level of PTHrP mRNA expression, and this is very tightly regulated, as might well be expected for a predominantly constitutive product with powerful local regulatory effects.[4]

The *PTHrP* gene and its products also have several levels of structural complexity that do not apply to the *PTH* gene and its product. The human *PTHrP* gene encodes three alternatively spliced isoforms, of as yet unknown biological significance. In addition, the primary PTHrP translation product(s) is subject to cleavage and processing into N-terminal, mid-region, and C-terminal products. There is also a functional nuclear localization sequence (NLS) at approximately the junction of the proximal two thirds and distal one third of the molecule.[4–6] The N-terminal and mid-region products and the NLS-bearing products have clear functional correlates, as described below.

PTHrP FUNCTIONS

By the early 1990s, it had become evident that PTHrP must be a local regulatory molecule, and the widespread expression of the gene in fetal tissues led many to suppose that it might function in development. This supposition led several groups to turn to gene manipulation techniques in mice, and the results of these experiments provided convincing evidence that PTHrP functions as a developmental regulatory molecule. These functions include such diverse processes as control of the formation of endochondral bone, the development of the mammary epithelium, and the eruption of teeth.[7–10] In the adult, the best-studied PTHrP function is regulation of the tone of accommodative smooth muscle structures such as the uterus, bladder, and vasculature, in which the *PTHrP* gene is induced by mechanical stretch and serves to relax the smooth muscle structure in question.[11,12]

The authors have reported no conflicts of interest.

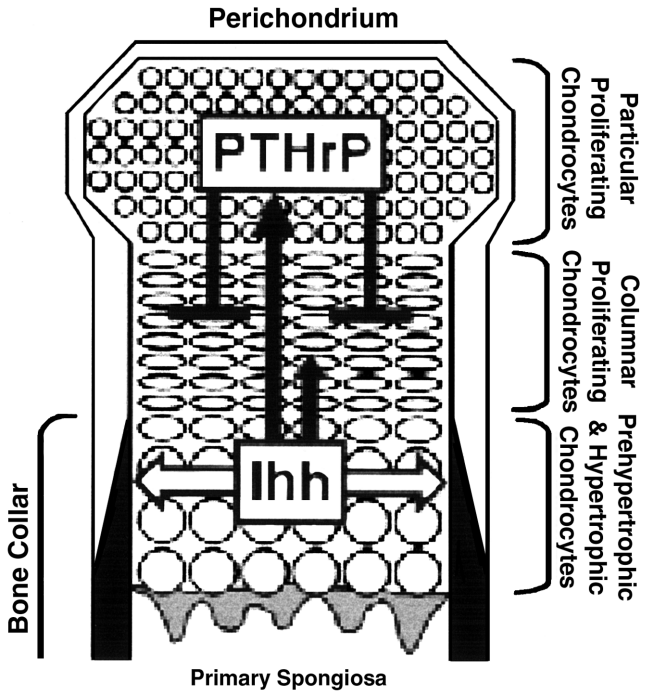

FIG. 1. PTHrP function in the cartilaginous growth plate. The chondrocyte differentiation program proceeds from undifferentiated chondrocytes above, through the proliferative chondrocytes in the columns, to the prehypertrophic and terminally differentiated hypertrophic chondrocytes below. PTHrP is expressed most abundantly in the proliferative chondrocytes at the junction of the periarticular and columnar populations and serves as a brake on the differentiation of the columnar chondrocytes to the hypertrophic stage. Indian hedgehog (Ihh) is produced by the chondrocytes as they hypertrophy and feeds back to increase PTHrP production, thereby slowing the rate at which the chondrocyte differentiation program proceeds. Absent PTHrP or PTH1R, the chondrocyte program proceeds too rapidly; the columns collapse, and premature mineralization and dwarfing take place. (Reproduced with permission from Chung U, Schipani E, McMahon AP, Kronenberg HM 2001 Indian hedgehog couples chondrogenesis to osteogenesis in endochondral bone development. J Clin Inv **107:**295–304.)

The Skeleton

Cartilage. At the time of its discovery, PTHrP represented a molecule in search of a function. The most powerful strategy for posing such a question is to knockout the gene in a mouse, and this experiment was reported in 1994.[7] The PTHrP-null mouse was something of a good news–bad news venture, the good news being that the mouse had a clear phenotype and the bad that this phenotype was a neonatal lethal.[7] Endochondral bone develops through a two-step process, the first step being the formation of a cartilaginous mold of the bone and the second a gradual replacement of the cartilage by bone; the most important biological consequence of this developmental strategy is the formation of the cartilaginous growth plate that provides the capacity for linear growth. The critical control point in this process is the chondrocyte differentiation program, a classical example of such a developmental program. In this program, chondrocytes progress through a series of stages to become terminally differentiated hypertrophic chondrocytes, which are mineralizing and apoptotic cells (Fig. 1). PTHrP normally regulates the rate at which this program proceeds, and its deletion results in a too rapid progression of the program that is associated with a classic chondrodysplasia (short-limbed dwarfism) that is lethal at birth.[7]

The details of PTHrP regulation and signaling in the growth plate are better understood than any other PTHrP regulatory function. PTHrP serves as the regulatory instrument of a higher-order developmental regulatory gene known as Indian hedgehog (Ihh), which is responsible for a number of different aspects of growth plate and bone cell development. This regulation functions as a classical negative feedback loop, with Ihh being produced by differentiating hypertrophic chondrocytes and feeding back through PTHrP to regulate the rate of chondrocyte differentiation through its differentiation program (Fig. 1). Thus, an increase in hypertrophic chondrocytes begats an increase in Ihh signaling and a PTHrP-mediated slowing of chondrocyte differentiation, and vice versa.[8]

PTHrP is also expressed in other cartilaginous sites. One is in the perichondrium that surrounds the costal cartilage,[13] which normally remains cartilaginous and enables expansion of the chest wall during breathing. It is presumably the loss of this PTHrP source that is responsible for the shield chest that causes the neonatal death of the PTHrP-null mouse.[13] Another site is the subarticular chondrocyte population just subjacent to the hyaline chondrocytes of the joint space.[13] PTHrP function here is unknown but might well be to prevent chondrocyte hypertrophy and mineralization of the joint space.

Bone. The abnormalities in the endochondral skeleton of the PTHrP-null mouse are seen as entirely a consequence of the absence of PTHrP regulation of chondrocyte development.[7,8] It was subsequently reported that heterozygous PTHrP-null mice are normal at birth but acquire trabecular osteopenia in their long bones at and beyond 3 months of age.[14] This observation has been recently confirmed in more detail as well as extended by the demonstration that conditional deletion of PTHrP in osteoblasts results in the same osteopenic phenotype.[15] In both models, bone formation and mineral apposition are reduced, as are the formation and survival of osteoblasts.[15] There is actually disagreement as to whether and/or in which osteoblast populations PTHrP is expressed, perhaps because of the use of different localization techniques or species/strains.[13–15] Nevertheless, the osteopenia in the two mouse models is intriguing and has led the authors to suggest that PTH and PTHrP may have quite distinct domains of influence regarding trabecular bone, PTHrP being a locally produced anabolic stimulus and PTH being a circulating resorptive agent charged with maintaining systemic mineral homeostasis.[15]

It turns out that PTHrP is also expressed in the periosteum and in the insertion sites of tendons and ligaments into cortical bone.[13] Periosteal bone formation is responsible for the accretion of bone at its periphery, a necessary consequence of bone growth as well as a compensatory mechanism to maintain skeletal strength when trabecular and/or endosteal bone is lost.[16] Tendon and ligament insertion sites are cemented in place by fibrous and/or fibrocartilaginous structures that are incorporated into mature cortical bone.[17] In certain of these sites, such as the tibial insertion of the medial collateral ligament, the insertion sites must migrate as the bone lengthens.[18] In all of these locations, PTHrP is not expressed in the underlying osteoblastic or bone cell populations but rather in the connective tissue cells in the outer layer of the periosteum or their equivalent at the myotendinous junction of the insertion sites. The function(s) of PTHrP in these locations is unknown, but the working hypotheses are that PTHrP may be induced in these sites by mechanical force[19] and serve to regulate localized bone formation and/or bone turnover.[13]

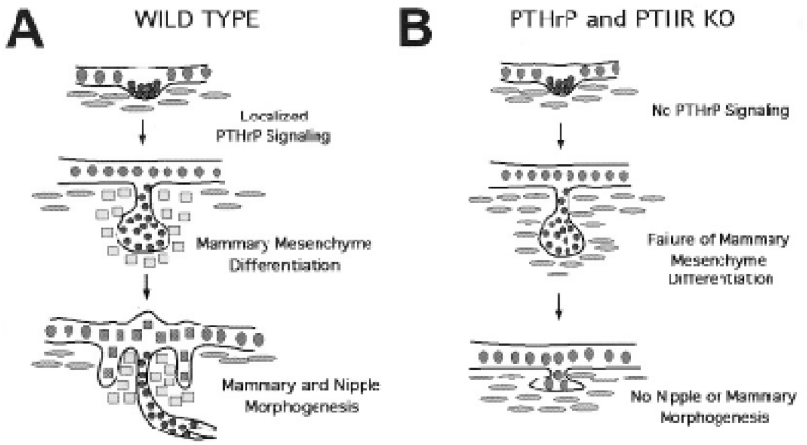

FIG. 2. PTHrP function in embryonic mammary epithelial development. (A) Normal mammary epithelial development, in which PTHrP (black dots) is expressed initially in an epidermal placode (top), subsequently in a bud (middle), and finally in branching epithelia that invade the mammary fat pad (bottom). PTHrP from the placode signals to the subjacent undifferentiated dermal mesenchymal cells (gray cigar-like cells) and drives their differentiation into specialized mammary mesenchymal cells (gray boxes). The mammary mesenchyme directs the formation of the nipple as well as the branching morphogenesis of the mammary bud. (B) Pattern in PTHrP-null (or PTH1R-null) mice. Here, the epithelial–mesenchymal signaling cascade is absent; the mammary mesenchyme fails to form, and the nascent mammary bud is resorbed. (Adapted with permission from Foley J, Dann P, Hong J, Cosgrove J, Dreyer BE, Rimm D, Dunbar ME, Philbrick WM, Wysolmerski JJ 2001 Parathyroid hormone-related protein maintains mammary epithelial fate and triggers nipple skin differentiation during embryonic breast development. Development **128:**513–525 and the Company of Biologists Ltd.)

Development of the Mammary Epithelium and Teeth and Reproductive Functions of PTHrP

By targeting an activated PTH/PTHrP receptor (PTH1R) or PTHrP itself to proliferative chondrocytes, it proved possible to "rescue" the PTHrP-knockout mouse from neonatal death.[9,10,20] In the PTHrP-targeted case, this generated a mouse that was PTHrP-sufficient in chondrocytes but PTHrP-null in all other sites.[9,10] These mice were small and fragile, but some survived and had a phenotype that in a human would be referred to as an ectodermal dysplasia. This phenotype included a failure of mammary epithelial development, a failure of tooth eruption, and abnormalities of a number of skin appendages such as the nails.[9,10,20–22] These structures share a common developmental history, each arising from a PTHrP-expressing epidermal placode/bud that signals the dermal mesenchyme to develop into a specialized connective tissue compartment that reciprocally drives the development of the epithelial structure in question. This kind of reciprocal paracrine signaling drives the formation of most epidermal appendages and is referred to as epithelial-mesenchymal morphogenesis. Many structures that form in this fashion continue their development postnatally, two cases in point being the mammary gland and the tooth.

Clearly, each of the abnormalities in the PTHrP-knockout and rescued PTHrP-knockout mice bespeaks a normal physiological function of PTHrP, and it would be presumed that a human syndrome that resulted from absent PTHrP signaling would be lethal and also would suffer from the combined abnormalities seen in these two mice. This proved to be the case in the form of Blomstrand chondrodysplasia, a rare and lethal syndrome that results from an inactivating mutation of the PTH1R and has a phenotype comprising the chondrodysplastic, mammary, and tooth impaction abnormalities seen in the mice.[23]

Mammary Gland. As just noted, mammary gland development begins early in embryonic life as a placode and then an inward bud of PTHrP-expressing epithelium that undergoes only limited development during fetal life. The adult mammary gland does not fully develop until adolescence, and it doesn't undergo full functional development until lactogenesis. It turns out that PTHrP has a number of regulatory functions in the mammary gland at several distinct stages of its development and function.

During embryonic development, PTHrP derived from the mammary bud "instructs" the dermal mesenchymal cells to differentiate into a specialized subjacent mammary mesenchyme[9,22] (Fig. 2). The mammary mesenchyme supports the growth and branching of the bud into the mammary fat pad in which it forms the pre-adolescent mammary epithelial tree that is present at birth.[9,22] In the absence of PTHrP (or its receptor), the mammary mesenchyme does not differentiate; the mammary bud degenerates, and no mammary epithelial structures remain.

At adolescence, the immature mammary epithelia develops into the adult gland by a process of branching morphogenesis.[22] This process is driven by PTHrP-expressing cutting cones known as end buds that penetrate and branch through the PTH1R-expressing stroma until they reach the margins of the mammary fat pad, which defines the limits of the adult mammary gland. Excess PTHrP actually inhibits branching morphogenesis, and it is thought that PTHrP therefore may regulate the spacing of the branches, but the details of how PTHrP functions during adolescent mammary development are not fully understood.

Lactation places an enormous demand on maternal calcium homeostasis: in rodents, up to one third of maternal skeletal mineral is transferred to the offspring in a 3-week period.[22] It now seems that PTHrP derived from the mammary epithelium drives the mobilization of bone mineral that feeds this process. Furthermore, the mammary epithelium is capable of sensing the serum calcium concentration through the calcium receptor and secretes PTHrP accordingly, much as would a parathyroid chief cell.[24] This is proposed to function as a mammary bone feedback loop and may be the only circumstance in which PTHrP normally circulates. Examples of pregnancy/lactation-associated hypercalcemia caused by PTHrP have been described but are very rare.[5] PTHrP is found at very high concentrations in breast milk (20–50 nM, some three orders of magnitude higher than its circulating concentration in patients

with humoral hypercalcemia of malignancy [HHM]), but its function in milk, if any, is unknown.[25]

Teeth. Teeth also develop from a PTHrP-expressing epithelial bud. By about embryonic day 14 in a mouse, the tooth has become encased in alveolar bone in what is aptly referred to as a dental crypt, from which it must escape. The process of tooth eruption is a remarkable example of uncoupled bone turnover in which osteoclasts form over the crown of the tooth and proceed to carve out the pathway through which the tooth will escape; bone formation at the base of the tooth subsequently propels it into the oral cavity.

It turns out that the teeth form quite normally in the rescued PTHrP-null mouse and that it is tooth eruption that fails.[10] The epithelial structure (known as the stellate reticulum) that surrounds the tooth in the crypt normally expresses abundant PTHrP just before eruption, which signals through connective tissue cells in the contiguous dental follicle to drive the formation of the crypt osteoclasts that sculpt the eruption pathway.[10,26] In the absence of PTHrP, the crypt osteoclasts do not form, and the tooth becomes progressively impacted in the crypt by encroaching alveolar bone.

The mammary epithelium and stellate reticulum are rapidly proliferating epithelial structures at the times when they deliver PTHrP into their developing microenvironments. Keratin-14 (K14) is a so-called proliferative keratin that also is expressed in proliferative epithelium. The K14 promoter was used to deliver PTHrP into the mammary epithelium and stellate reticulum in a second-generation crossing experiment that successfully rescued embryonic mammary epithelial development and also triggered tooth eruption.[9,10,22] This experiment reaffirmed the capacity of PTHrP from these epithelial structures to drive the developmental programs in question.

Skin. Human keratinocytes in primary culture were the first normal cells shown to express PTHrP, the lead here being the frequency of squamous cell carcinomas among patients with HHM.[27] While the functions of PTHrP in ectodermal derivatives such as the mammary gland and tooth are clear, there is disagreement as to what the function(s) of PTHrP might be in regulating the development/growth of hair and other epidermal appendages and, indeed, whether PTHrP is actually expressed in the interfollicular epidermis in vivo.[4,5,19,21,28]

Placental Calcium Transport. To subserve fetal mineral requirements, calcium is transported across the placenta through a pump that maintains a maternal–fetal gradient (i.e., the ambient calcium concentration is higher in the fetus). Regulation of placental calcium transport by PTHrP was initially suggested by the Melbourne group.[29] Physiological studies of this question have been carried out in sheep[29,30]and genetic studies in mice.[31,32] The gradient is lost in PTHrP-null mice and is restored by infusion of a mid-region fragment of PTHrP but not by an N-terminal fragment or PTH itself.[31] The same mid-region specificity has been shown in sheep.[30] There is general agreement that the placenta itself is a major source of the PTHrP[29–32] and that the calcium receptor is probably involved in gating placental PTHrP production.[22] A mid-region PTHrP receptor has yet to be identified.

PTHrP Expression and Function in Excitable Cells

In 1925, crude parathyroid extracts were infused into dogs and were found to decrease systemic blood pressure.[33] This triggered an interest in the putative regulation by PTH of smooth muscle, the heart, and neurons that persisted for ~60 years.[12] There was never a physiological rationale for such regulatory effects, in that PTH release from the parathyroid chief cell is gated by the ambient ionized calcium concentration in a classic negative feedback loop, and it circulates at some 1/1000th the concentration that these effects require. It now seems clear that PTHrP is the natural ligand that mediates such effects and that this regulation is local rather than systemic.

Smooth Muscle and the Cardiovascular System. PTHrP expression and regulation of smooth muscle cells can be summarized briefly as follows: (1) the PTHrP gene seems to be expressed by every smooth muscle cell/bed in the body; (2) in most if not all of these sites PTHrP is induced by mechanical stretch; (3) PTHrP effects are autocrine or paracrine and are mediated by the PTH1R; and (4) PTHrP functions to relax the smooth muscle cells or structure in question.[11,12] In the vasculature, PTHrP is also induced by vasoconstrictive agents such as angiotensin II and seems to have its principal effects on resistance vessels.[12] In general, PTHrP is seen as a local modulator of smooth muscle tone in specific vascular beds rather than as a systemic regulatory factor.[12,34] In accommodative smooth muscle structures such as the stomach, bladder, and uterus, stretch-induced PTHrP-driven smooth muscle relaxation allows the structure to accommodate gradual filling.[11,12] This can be shown in the uterus by simply inflating a balloon in one horn of the bicornuate rodent uterus. However, the major physiological peak in PTHrP expression in the uterus occurs at parturition,[11] and its exact role in the yin-yang control of smooth muscle function during this time is not understood.

PTHrP is also expressed in endothelial cells.[34–36] Several studies have shown that PTHrP can have potent antiangiogenic effects, which seem to be mediated by a combination of endothelial and smooth muscle actions.[35,36] Another recent series of experiments in rats revealed that PTHrP could be induced in the endothelium of cerebral microvessels by ischemia and that the vasodilatation induced by PTHrP downstream could limit the size of a cerebral infarct in a stroke model system.[37]

In isolated hearts, PTHrP has both positive ionotropic and chronotropic effects, but the ionotropy is thought to be secondary to a vasodilatation-induced increase in coronary flow.[12] PTHrP has been colocalized with atrial natriuretic peptide–containing granules in the rat atrium, but the functional implications of this finding are unknown.[12]

Central and Peripheral Nervous Systems. Both PTHrP and PTH1R are widely expressed in neurons of the cerebral cortex, hippocampus, and cerebellum.[38,39] Many of these PTHrP-expressing neuronal populations have in common abundant expression of excitatory amino acid (glutamate) receptors and L-type calcium channels and a known propensity to excitotoxicity, a form of neuronal damage that results from excessive excitation. In cerebellar granule cells, PTHrP expression is induced by depolarization-driven L channel calcium influx and seems to be capable of feeding back through the PTH1R to dampen L channel activity in an autocrine/paracrine fashion.[40] The net result is a neuroprotective feedback loop capable of combating excessive calcium-associated excitotoxicity.[40] The rescued PTHrP-null mouse displays an enhanced sensitivity to excitotoxic stimuli in vivo but is sufficiently fragile that it is not an ideal model system for such studies.[40]

PTHrP and the PTH1R are also expressed in neurons and glia of the peripheral nervous system (PNS). After peripheral nerve crush or sectioning, Schwann cells dedifferentiate, proliferate, and provide both a conduit and growth factors that enable axonal regeneration to occur.[41] PTHrP expression is

dramatically increased in these dedifferentiated Schwann cells after injury and seems to retard the Schwann cell differentiation program much as it does the chondrocyte differentiation program, maintaining the immature Schwann cell population that is critical to PNS regeneration.

Pancreatic Islets. PTHrP is expressed in all four cell types of the pancreatic islet, most particularly the β cell.[22] β cells are classic neuroendocrine cells, in that they secrete insulin but are gated to do so electrophysiologically, the proximate secretory signal being L channel–mediated calcium influx. PTHrP is stored and cosecreted with insulin, but PTHrP does not seem to feed back on β-cell L channels as it does in some neurons (C. Macica, unpublished results, 2003). Overexpression of PTHrP in the β cells of transgenic mice is associated with an increased β-cell mass together with hyperinsulinemia and hypoglycemia,[42] but there is no obvious islet phenotype in the PTHrP-knockout or rescued PTHrP-knockout mouse.[22]

MECHANISM OF ACTION OF PTHrP

The discovery of PTHrP as the major mediator of HHM was based on its ability to replicate the actions of PTH on the PTH1R.[1,3,43] Indeed, most patients with HHM display elevated urinary excretion of nephrogenous cAMP,[44] reflecting the action of systemic PTHrP to stimulate renal adenylyl cyclase through the PTH1R. Purified PTHrP and synthetic amino-terminal fragments of PTHrP display an affinity for the PTH1R that is comparable with that of PTH.[45,46] In patients with HHM, the ability of tumor-derived PTHrP to mimic the skeletal and renal effects of excess PTH is attributable to PTHrP-induced activation of PTH1R signaling.

The physiological effects of PTHrP as a paracrine factor are also mediated, at least in part, by PTH1R signaling. Mice and humans with deletion of the PTH1R display chondrogenic defects that resemble those produced by PTHrP deletion in the mouse.[47] Null mutations in both genes produce neonatal death, but the chondrogenic defect in PTH1R-null mice is more severe than that seen in PTHrP-null mice. This suggests that a PTH1R ligand(s) other than PTHrP might contribute to the control of chondrocyte differentiation, at least in the absence of PTHrP. cAMP signaling seems to be critical for the actions of PTHrP to suppress chondrocyte maturation.[48] In support of this, deletion of the α-subunit of Gs (the G protein that couples the PTH1R to adenylyl cyclase) in chondrocytes was shown to phenocopy the effects of deletion of PTHrP.[49,50] This provides direct experimental evidence that cAMP signaling plays a central role in mediating the effects of PTHrP on cartilage development. cAMP signaling through the PTH1R is likely to be important for other physiological actions of PTHrP as well. For example, increased levels of cAMP reduce smooth muscle tone, and this mechanism almost certainly is essential for the actions of PTHrP as a paracrine smooth muscle relaxant.

Understanding PTHrP action is complicated by the fact that multiple forms of the peptide are synthesized by alternative splicing and that additional fragments arise from post-translational processing.[51,52] Three synthesized forms have been identified, and these consist of 139, 141, and 173 amino acids. These forms are identical through amino acid 139, and thus contain the same critical 1–34 sequence required for binding with high affinity to the PTH1R. Indeed, eight of the amino terminal residues of PTHrP are identical with the corresponding residues in PTH, and this underlies the ability of these two peptides to activate a common receptor (PTH1R). Different tissues and tumors display different patterns of expression of these secretory isoforms, but the biological significance of this remains obscure. The isoforms of PTHrP are cleaved by prohormone convertases, resulting in the production of multiple secreted peptides.[53–56] Cleavage products include an amino-terminal fragment that retains the ability to bind to and activate the PTH1R; a mid-region PTHrP fragment that may have biological actions that are distinct from those of intact or amino-terminal PTHrP (e.g., stimulation of placental calcium transport) and carboxy-terminal fragments that are detectable in the circulation. The functional importance of carboxy-terminal PTHrP peptides is not well defined. Such peptides are capable of producing calcium transients in hippocampal neurons,[57] and in some systems, produce inhibition of bone resorption.[58,59] These findings have led to the concept that PTHrP may be a polyhormone (i.e., the precursor of multiple biologically active peptides).[60]

In the arterial wall, PTHrP is expressed in proliferating vascular smooth muscle cells in culture and after balloon angioplasty in vivo.[61] The level of PTHrP is increased in atherosclerotic coronary arteries.[62] Exposure of rat vascular smooth muscle cells to PTHrP has an antimitotic effect, suggesting that locally released PTHrP would act to inhibit the response to a proliferative stimulus.[63] In contrast, when transfected into A10 rat vascular smooth muscle cells, PTHrP induces marked proliferation.[64] The proliferative response does not occur with transfection of mutant forms of PTHrP from which polybasic amino acid sequences between residues 88 and 106 had been deleted. These sequences have been shown to function as a nuclear localization sequence in other cells.[6] Wildtype PTHrP is targeted to the nucleus of A10 cells, but the deletion mutants fail to localize to the nucleus. Nuclear localization of PTHrP seems to require association of the peptide with importin-β.[65] Interestingly, a leucine-rich sequence in the C-terminal region of PTHrP seems to regulate nuclear export of the protein.[66] It has therefore been postulated that in addition to binding to cell surface receptors, PTHrP can have direct nuclear actions, termed intracrine actions. Because secreted fragments of PTHrP and its intracrine actions seem to have opposing effects on proliferation, PTHrP could interplay in a complex fashion with other proliferative factors in determining the response of the vascular wall to injury or atherosclerosis.

Potential intracrine actions of PTHrP have recently been shown to extend beyond vascular smooth muscle cells.[67,68] It is possible that this represents a major alternate signaling pathway for PTHrP in addition to G-protein signaling through the PTH1R, although this has yet to be definitively established. Intracrine signaling by PTHrP can occur through multiple mechanisms.[69] In some cases, secreted PTHrP may be taken up by cells through endocytosis, with subsequent trafficking of the protein to the nucleus.[70,71] It is not clear whether this endocytosis requires the binding of PTHrP to the PTH1R. A second possibility is that initiation of translation of PTHrP mRNA may occur through an alternative start site, resulting in the synthesis of a cytoplasmic form of the peptide capable of entering the nucleus.[72] Finally, PTHrP may be transported in retrograde fashion from the endoplasmic reticulum to the cytoplasm.[73] The nuclear localization of PTHrP seems to be regulated by phosphorylation of the protein, with PTHrP appearing either in the nuclear matrix or in the nucleolus, depending on the system.[70] In some cases, nuclear localization of PTHrP varies markedly as a function of the progression of cells through the cell cycle, suggesting a functional role of PTHrP in cell proliferation and/or survival.[74] Indeed, there is evidence that the intracrine actions of PTHrP can promote cell proliferation or survival, depending on the cell type.[6,75,76] Nuclear PTHrP may also function as a regulator of gene expression, as has recently been suggested in prostate cancer

cells.[77] Additional studies are needed to determine how intracrine signaling cooperates with classical PTH1R signaling in mediating the pleiotropic biological effects of PTHrP.

CONCLUSIONS

PTHrP was isolated and cloned from human tumors associated with the syndrome of HHM in the late 1980s. PTHrP is not the first physiologically relevant peptide to have been found in such a bad neighborhood, a previous example being the isolation of growth hormone releasing hormone (GHRH) from a human pancreatic carcinoma by three independent groups in 1982.[78] The principal difference in the GHRH and the PTHrP stories was the outcome: GHRH already had a well-recognized job description before it was defined structurally in 1982, whereas PTHrP was identified in structural terms in 1987 well before any of its biological functions were recognized.

PTHrP is now known to have a variety of functions during both fetal and adult life. Among these one can begin to recognize several common themes: (1) virtually all such PTHrP effects involve paracrine pathways; (2) a number of these constitute classical epithelial–mesenchymal cascades; (3) in several sites, PTHrP seems to control the rate in which programs of differentiation proceed; (4) in several sites (e.g., the tooth and mammary bud), PTHrP serves to regulate the migration of an epithelial structure through a connective tissue/mesenchymal compartment; (5) in a number of systems (e.g., the growth plate, mammary epithelium and tooth), PTHrP regulates developmental events that occur and/or continue postnatally; (6) in several types (smooth muscle and some bone cells), PTHrP is mechanically induced; and (7) in a number of locations (e.g., placenta, the dental crypt, cartilage, and bone), PTHrP actions are calcium-related to one or another extent.

In the end, it is not so surprising that a constitutive secretory product that is widely expressed, particularly in proliferative epithelia, might turn out to be the mediator of a common "tumor-humor" syndrome.

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Chapter 17. Vitamin D: Photobiology, Metabolism, Mechanism of Action, and Clinical Applications

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INTRODUCTION

Vitamin D is a secosteroid that is made in the skin by the action of sunlight.[1] Vitamin D (D represents either or both D_2 and D_3) is biologically inert and must undergo two successive hydroxylations in the liver and kidney to become the biologically active 1,25-dihydroxyvitamin D [$1,25(OH)_2D$].[1–4] $1,25(OH)_2D$'s main biological effect is to maintain the serum calcium within the normal range. It accomplishes this by increasing the efficiency of intestinal absorption of dietary calcium and by recruiting stem cells in the bone to become mature osteoclasts, which, in turn, mobilizes calcium stores from the bone into the circulation.[1–4] The renal production of $1,25(OH)_2D$ is tightly regulated by serum calcium levels and through the action of PTH and phosphorus (Fig. 1). There are a wide variety of inborn and acquired disorders in the metabolism of vitamin D that can lead to both hypo- and hypercalcemic conditions. $1,25(OH)_2D$ not only regulates calcium metabolism but also is capable of inhibiting the proliferation and inducing terminal differentiation of a variety of normal and cancer cells, modulating the immune system, enhancing insulin secretion, and downregulating the renin/angiotensin system.[1,2] Active vitamin D compounds are used for the treatment of osteoporosis, renal osteodystrophy, and psoriasis and are being developed to treat some cancers, hypertension, benign prostate hypertrophy, cardiovascular heart disease, and type I diabetes.[1–6]

PHOTOBIOLOGY OF VITAMIN D_3

During exposure to sunlight, cutaneous 7-dehydrocholesterol (7-DHC; provitamin D_3), the immediate precursor of cholesterol, absorbs solar radiation with energies between 290 and 315 nm (ultraviolet B [UVB]), transforming it into previtamin D_3 (Fig. 1).[1,2] Once formed, previtamin D_3 rapidly undergoes a membrane enhanced temperature dependent isomerization to vitamin D_3 (Fig. 1).[2] Vitamin D_3 is translocated from the skin into the circulation, where it is bound to the vitamin D–binding protein (DBP).[1,2]

There are no documented cases of vitamin intoxication caused by excessive exposure to sunlight, because once previtamin D_3 and vitamin D_3 are formed, they absorb solar UVB radiation and are transformed into several biologically inert photoproducts (Fig. 1).[1,2]

A variety of factors can alter the cutaneous production of vitamin D_3. Melanin, an excellent natural sunscreen, competes with 7-DHC for UVB photons, thereby reducing the production of vitamin D_3.[1,2] People of color require longer exposure (5- to 10-fold) to sunlight to make the same amount of vitamin D_3 as their white counterparts.[1,2] Aging diminishes the concentration of 7-DHC in the epidermis. Compared with a young adult, a person over the age of 70 years produced $<30\%$ of the amount of vitamin D_3 when exposed to the same amount of simulated sunlight.[1,2] Latitude, time of day, and season of the year dramatically affect the production of vitamin D_3 in the skin (Fig. 2). At latitudes of 42° N (Boston) and 48.5° N (Paris, France) or 52° N (Edmonton, Canada), sunlight is incapable of producing vitamin D_3 in the skin between the months of November through February and October through March,[1,2] respectively. Casual exposure to sunlight provides most (80–100%) of our vitamin D requirement. The inability of the sun to produce vitamin D_3 in the far northern and southern latitudes during the winter requires both children and adults to take a vitamin D supplement to prevent vitamin D deficiency. For children and young adults, the cutaneous production of vitamin D_3 during the spring, summer, and fall is often in excess and is stored in the fat so that it can be used during the winter months. However, both children and adults who always wear sun protection may not make enough vitamin D_3 and therefore do not have sufficient vitamin D stores for winter use and will become vitamin D deficient.[1] Exposure to sunlight at lower latitudes such as Los Angeles (24° N), Puerto Rico (18° N), and Buenos Aires (34° S) results in the cutaneous production of vitamin D_3 during the entire year (Fig. 2).[1,2] A sunscreen with a sun protection factor of 8 (SPF 8) reduced the cutaneous production of vitamin D_3 by 95%.[1,2] Chronic use of a sunscreen can result in vitamin D insufficiency.[1,2] Although sunscreen use is extremely valuable for the prevention of skin cancer and the damaging effects caused by excessive exposure to the sun, both children and adults who depend on sunlight for their vitamin D_3 should consider exposure of hands, face, and arms or arms and legs to suberythemal amounts of sunlight (25% of the amount that would cause a mild pinkness to the skin) two to three times a week before topically applying a sunscreen with an SPF of 15. Thus, they can take advantage of the beneficial

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effect of sunlight while preventing the damaging effects of chronic excessive exposure to sunlight.

FOOD SOURCES AND THE RECOMMENDED ADEQUATE INTAKE FOR VITAMIN D

Vitamin D is rare in foods. The major natural sources of vitamin D are oily fish such as salmon and mackerel (~400 IU/3.5 oz) as well as fish liver oils including cod liver oil.[1–3] Vitamin D can also be obtained from foods fortified with vitamin D including some cereals, bread products, yogurt, orange juice, and milk.[1,7] Other dairy products including ice cream and cheese are not fortified with vitamin D. In Europe, most countries except Sweden forbid vitamin D fortification of milk. However, margarine and some cereals are fortified with vitamin D in most European countries. Multivitamin and pharmaceutical preparations containing vitamin D are reliable sources of vitamin D. The new recommended adequate intake (AI) for vitamin D for infants, all children, and adults up to the age of 50 years is 200 IU (5 μg)/day. For adults 51–70 and 71+ years, the AIs are 400 and 600 IU/day, respectively.[7] There is mounting evidence that, in the absence of sunlight, the recommended AIs for vitamin D_3 for children and adults is inadequate without some sun exposure and they may need as much as 1000 IU of vitamin D_3 a day.[8–14]

METABOLISM OF VITAMIN D

Vitamin D_2, which comes from yeast and plants, and vitamin D_3, which is found in oily fish and cod liver oil and is made in the skin, are the major sources of vitamin D.[1,2,7] The differences between vitamin D_2 and vitamin D_3 are a double bond between C_{22} and C_{23}, and a methyl group on C_{24} for vitamin D_2. Vitamin D_2 is about 30% as effective as vitamin D_3 in maintaining vitamin D status.[13,14] Once vitamin D_2 or vitamin D_3 enters the circulation, it is bound to the vitamin D–binding protein and transported to the liver, where one or more cytochrome P_{450}-vitamin D-25-hydroxylase(s) (CYP27A1, CYP3A4, CYP2R1, CYP2J3) introduces a OH on carbon 25 to produce 25-hydroxyvitamin D [25(OH)D](Fig. 1).[1–3,10] 25(OH)D is the major circulating form of vitamin D. Because the hepatic vitamin D-25-hydroxylase is not tightly regulated, an increase in the cutaneous production of vitamin D_3 or ingestion of vitamin D will result in an increase in circulating levels of 25(OH)D.[1–3,7,10,14] Therefore, its measurement is used to determine whether a patient is vitamin D deficient, sufficient, or intoxicated.[1–3,7,10,14]

25(OH)D is biologically inert. It is transported to the kidney, where membrane-bound megalin transports the 25(OH)D-DBP complex into the renal tubule cell where the cytochrome P_{450}-mono-oxygenase, 25(OH)D-1α-hydroxylase (1-OHase; CYP27B1), metabolizes 25(OH)D to 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] (Fig. 1).[1–4] Although the kidney is the major source of circulating $1,25(OH)_2D$, there is strong evidence that a wide variety of tissues and cells including activated macrophages, osteoblasts, keratinocytes, prostate, colon, and breast express the 1-OHase and have the ability to produce $1,25(OH)_2D$.[1–3,15] In addition, during pregnancy, the placenta produces $1,25(OH)_2D$.[3] However, because anephric patients have very low or undetectable levels of $1,25(OH)_2D$ in their blood, the extrarenal sites of $1,25(OH)_2D$ production do not seem to play a role in calcium homeostasis. The exception is when macrophages make and excrete $1,25(OH)_2D$ into the circulation in patients with chronic granulomatous disorders such as sarcoidosis. This results in hypercalciuria and hypercalcemia.[3,15] The local production of $1,25(OH)_2D$ in tissues not associated with calcium homeostasis may be for the purpose of regulating a wide variety of biological functions including cell growth, apoptosis, angiogenesis, differentiation, and regulation of the immune system.[1–6,15]

When serum ionized calcium declines, there is an increase in the production and secretion of PTH. PTH has a variety of biological functions on calcium metabolism. It also regulates calcium homeostasis by enhancing the renal conversion of 25(OH)D to $1,25(OH)_2D$ (Fig. 1).[1–3] It does this by acting on the 1-OHase promoter and indirectly through its renal wasting of phosphorus resulting in decreased intracellular and blood levels of phosphorus.[1,3] Hypophosphatemia and hyperphosphatemia are associated with increased and decreased circulating concentrations of $1,25(OH)_2D$, respectively.[16] Fibroblast growth factor (FGF)-23 is involved in phosphorus metabolism and markedly reduces the kidney's production of $1,25(OH)_2D$.[17] Calcium deprivation will also enhance 1-OHase activity independent of the associated secondary hyperparathyroidism.[18] A variety of other hormones associated with growth and development of the skeleton or calcium regulation including growth hormone and prolactin indirectly increase the renal production of $1,25(OH)_2D$. IGF-1 is a potent stimulator of $1,25(OH)_2D$ production and may explain the parallelism between serum $1,25(OH)_2D$ levels and growth velocity in children and adolescents.[19] Osteoporotic patients may lose their ability to upregulate the renal production of $1,25(OH)_2D$ by PTH.[20] This, along with a decrease in the amount of vitamin D receptor in elders' small intestine,[21] may help explain the age-related decrease in the efficiency of intestinal calcium absorption.

Both 25(OH)D and $1,25(OH)_2D$ undergo a 24-hydroxylation by the 25(OH)D-24-hydroxylase (CYP24) to form 24,25-dihydroxyvitamin D [$24,25(OH)_2D$] and 1,24,25-trihydroxyvitamin D, respectively.[1–4] $1,25(OH)_2D$ undergoes several hydroxylations in the side-chain by the 25(OH)D-24-hydroxylase causing the cleavage of the side-chain between carbons 23 and 24, resulting in the biologically inert water soluble acid, calcitroic acid (Fig. 1).[1–4] Although >50 different metabolites of vitamin D have been identified, only $1,25(OH)_2D$ is believed to be important for most if not all of the biological actions of vitamin D on calcium and bone metabolism.[1–4]

A wide variety of drugs including antiseizure medications, glucocorticoids, and rifampin enhance the catabolism of vitamin D. It is now recognized that when the pregnane-X-receptor (PXR) binds any one of these drugs and becomes activated, it can form a heterodimeric complex with retinoic acid X receptor (RXR). This complex binds to the VDR responsive element (VDRE) of the CYP24 and enhances the expression of the 24-OHase and the catabolism of 25(OH)D and $1,25(OH)_2D$.[22] Thus, drugs and xenobiotics that activate PXR may also increase CYP24 activity, causing vitamin D deficiency and osteomalacia.

MOLECULAR BIOLOGY OF VITAMIN D

$1,25(OH)_2D$ is a steroid hormone and acts similar to estrogen and other steroid hormones in inducing its biological responses.[1–4,23] $1,25(OH)_2D$ binds to the VDR in the cytoplasm causing conformational change to reorient the activation function 2 domain located in helix 12 of the receptor. This allows for it to interact with other cytoplasmic proteins and co-activators, which mediates its translocation along the microtubule with the hydrophilic nuclear localization sequence binding to importin α to enter the nucleus through the nuclear pore complex.[23,24] In the nucleus, the VDR–$1,25(OH)_2D_3$ complex binds with RXR (Fig. 3). This heterodimeric complex binds to the VDRE and initiates the binding of several initiation

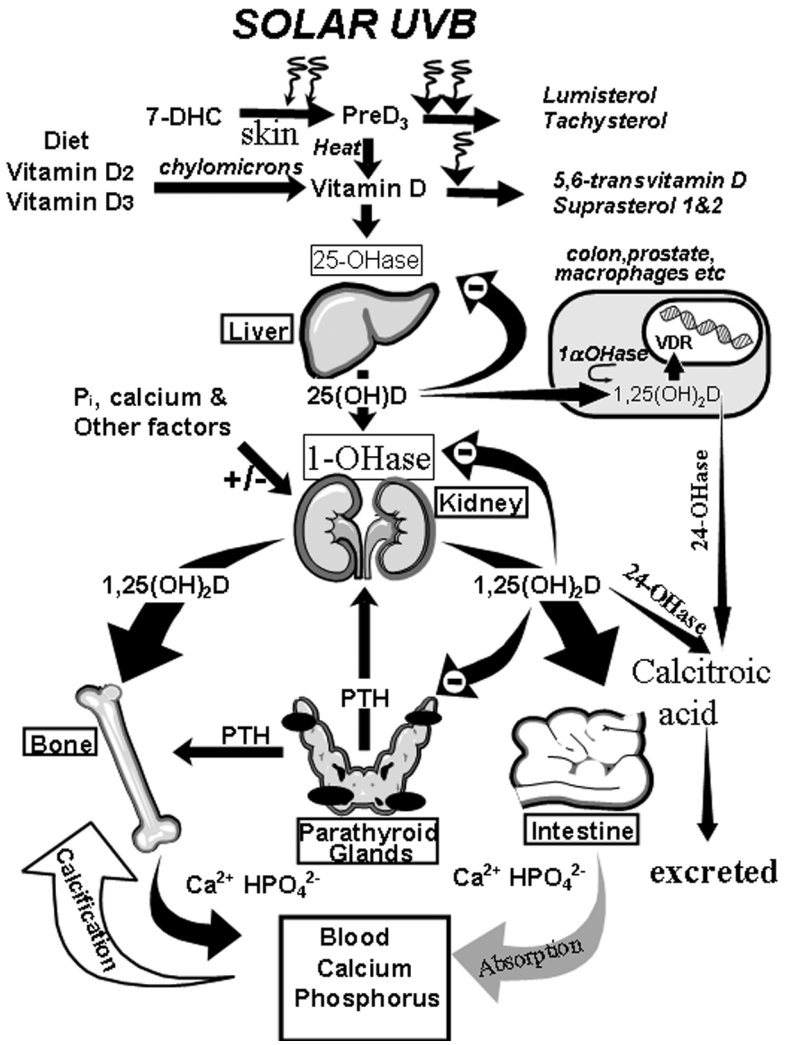

FIG. 1. The photochemical, thermal, and metabolic pathways for vitamin D. During exposure to sunlight, UVB 7-DHC is converted to previtamin D_3 (preD_3). PreD_3 undergoes thermal isomerization to vitamin D_3, and vitamin D from the diet, along with the skin's vitamin D, enters the circulation and is metabolized sequentially in the liver and kidney to 25(OH)D and $1,25(OH)_2D$. Serum phosphorus (Pi) and PTH levels are major regulators of renal $1,25(OH)_2D$ production. The 24-OHase is responsible for the degradation of $1,25(OH)_2D$ to calciotropic acid. 25(OH)D can also enter other nonrenal tissues including macrophages, colon, prostate, and breast, where it undergoes transformation to $1,25(OH)_2D$. It then interacts with its VDR to induce genes that regulate cell growth.

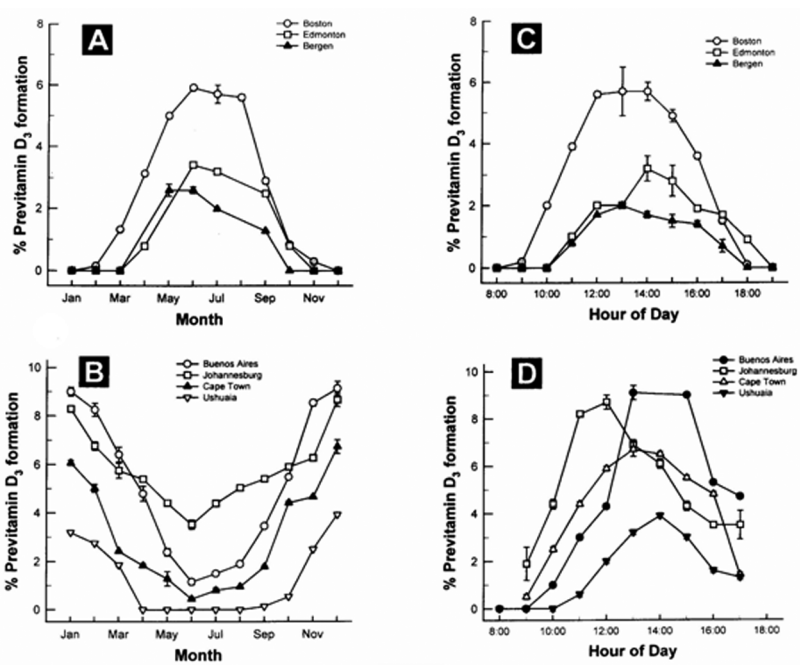

FIG. 2. Influence of season, time of day, and latitude on the synthesis of preD_3 in (A and C) the Northern and (B and D) Southern Hemispheres. The hour indicated in C and D is the end of the 1-h exposure time. Data for hour of the day were collected in July. Data represent means ± SE of duplicate determinations.

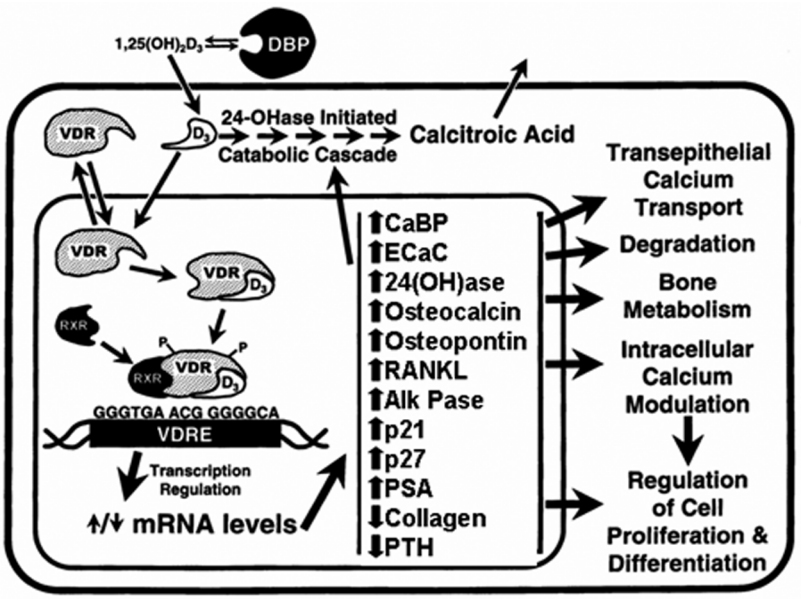

FIG. 3. A schematic representation of the mechanism of action of $1,25(OH)_2D$ in various target cells resulting in a variety of biological responses. The free form of $1,25(OH)_2D_3$ enters the target cell and interacts with its nuclear VDR, which is phosphorylated (Pi). The $1,25(OH)_2D$–VDR complex combines with the RXR to form a heterodimer, which in turn interacts with the VDRE, causing an enhancement or inhibition of transcription of vitamin D responsive genes including calcium-binding protein (CaBP), epithelial calcium channel (ECaC), 25(OH)D-24-hydroxylase (24-OHase), RANKL, alkaline phosphatase (alk PASE), prostate specific antogen (PSA), and PTH.

factors including the P160 co-activator proteins glucocorticoid receptor interacting protein 1 (GRIP-1), steroid receptor co-activator-1 (SRC-1), and vitamin D receptor interacting protein (DRIP)–thyroid receptor associated proteins (TRAP) complex along with a host of other coactivators that ultimately initiates transcription of the vitamin D responsive gene.[3,23]

The *VDR* gene has nine exons. The first exon includes several noncoding regions (Ia to If), whereas the eight following exons give rise to the classical VDR. Unlike other nuclear receptors of the same family, only a few isoforms of VDR have been identified, and their functional relevance remains unclear. Aside from the classical VDR, two N-terminal variances of the human VDR have been described. One is three amino acids shorter and is associated with the *Fok*I start codon polymorphism in the human VDR locus.[25] The other is 50 amino acids longer and results from the coding of the Id exon through alternative splicing.[26] Specific exon mutations and exon skipping have been identified, which caused resistance to $1,25(OH)_2D$, causing vitamin D–resistant rickets (also known as vitamin D–dependent rickets type 2).[25]

Some VDR actions may not require its binding to $1,25(OH)_2D$. When the ligand binding domain of the VDR was mutated so that it could no longer bind $1,25(OH)_2D_3$, but was able to still interact with the RXR and the VDRE maintaining hair follicle homeostasis.[27] VDR can also bind non–vitamin D ligands including lithocholic acid, which is hepatotoxic and a potential carcinogenic bile acid and thus may mediate local cellular detoxification through induction of the CYP3A4 expression.[28] $1,25(OH)_2D$ may also be recognized by other receptors than VDR including MARRS, a multifunctional membrane associated protein isolated from chick intestine that stimulated phosphate uptake.[29] The functional importance of the interaction of these non–vitamin D ligands with VDR, however, is not well understood.[30]

There are also mutations in the exons and introns that can lead to polymorphisms of the *VDR* gene that do not cause any biologically significant alteration in the amino acid composition of the VDR.[31] These polymorphisms are thought to be important in the transcription of the *VDR* gene and/or stabilization of the resultant VDR mRNA. There is some evidence that these polymorphisms may lead to a differential responsiveness to $1,25(OH)_2D_3$ in the intestine and bone, thereby playing a role in peak bone mass and the development and osteoporosis and other diseases.[32–34]

BIOLOGIC FUNCTIONS OF VITAMIN D IN THE INTESTINE AND BONE

The major physiologic function of vitamin D is to maintain serum calcium at a physiologically acceptable level to maximize a wide variety of metabolic functions, signal transduction, and neuromuscular activity.[1–3] It accomplishes this by interacting with its receptor in the small intestine. $1,25(OH)_2D$ enhances calcium entry by inducing the epithelial calcium channel (ECaC), a member of the vanillanoid receptor family (TRPV6).[3,35] $1,25(OH)_2D$ also induces several proteins in the small intestine, including calcium binding protein (calbindin D 9K), alkaline phosphatase, low affinity Ca ATPase, brush border actin, calmodulin, and several brush border proteins of 80–90 kDa.[3,35] These facilitate the movement of calcium through the cytoplasm and transfer the calcium across the basal lateral membrane into the circulation. $1,25(OH)_2D$ causes a biphasic response on intestinal calcium absorption in vitamin D–deficient animals. A rapid response occurs within 2 h and peaks by 6 h and another that begins after 12 h and peaks at 24 h, suggesting that there is a rapid action of $1,25(OH)_2D$ on intestinal calcium absorption and a more prolonged nuclear mediated response.[3,35] $1,25(OH)_2D$ also enhances the absorption of dietary phosphorus. Although calcium and phosphorus absorption occur along the entire length of the small intestine, most of the phosphorus transport occurs in the jejunum and ileum unlike calcium absorption, which principally occurs in the duodenum. The net result is that there is an increase in the efficiency of intestinal calcium and phosphorus absorption. In the vitamin D–deficient state, no more than 10–15% of dietary calcium and 60% of dietary phosphorus is absorbed the gastrointestinal tract. However, with adequate vitamin D, adults absorb 30–40% of dietary calcium and 70–80% of dietary phosphorus by the $1,25(OH)_2D$-mediated processes. During pregnancy and lactation and during the growth spurt, circulating concentrations of $1,25(OH)_2D$ increase, thereby increasing the efficiency of intestinal calcium absorption by as much as 50–80%.[1–3,35]

When there is inadequate dietary calcium to satisfy the

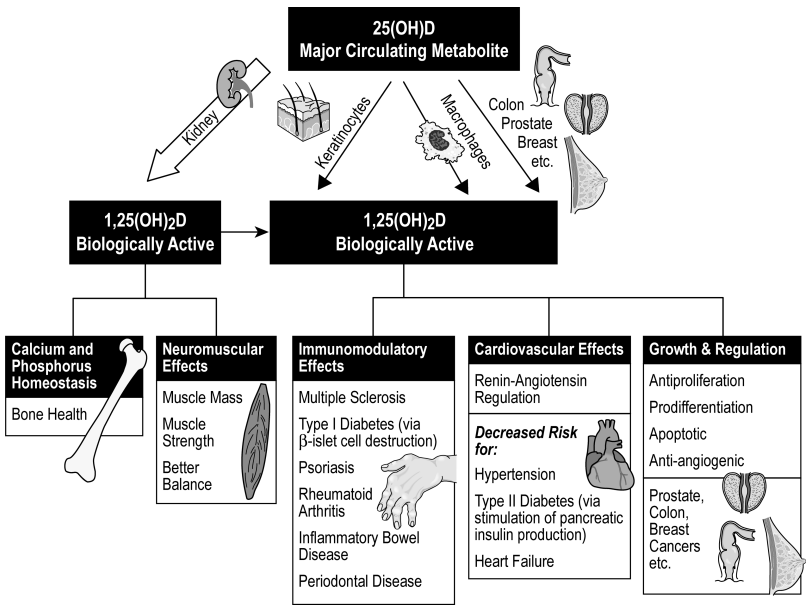

FIG. 4. Sources of vitamin D and its metabolism to $1,25(OH)_2D$ in the kidney and extrarenal tissues, including prostate gland, breast, colon, and immune cells. Once produced, $1,25(OH)_2D$ has a multitude of biological actions on calcium, muscle, and bone health, regulation of blood pressure and insulin production, regulation of immune function, and regulation of cell growth.

body's calcium requirement, $1,25(OH)_2D$ interacts with the VDR in osteoblasts resulting in signal transduction to induce RANKL expression. The pre-osteoclast has the receptor (RANK) for RANKL.[1–3,36,37] The direct contact of the pre-osteoclast's RANK with the osteoblast's RANKL results in signal transduction to induce pre-osteoclasts to become mature osteoclasts. The mature osteoclasts release hydrochloric acid and proteolytic enzymes to dissolve bone mineral and matrix releasing calcium into the extracellular space.

The major function of $1,25(OH)_2D$ for the bone mineralization process is to maintain a calcium-phosphorus product in the circulation that is in a supersaturated state, thereby resulting in the passive mineralization of the collagen matrix (osteoid) laid down by osteoblasts. $1,25(OH)_2D$ does not have a direct active role in the mineralization process; its responsibility is to maintain blood levels of calcium and phosphorus in the normal range for proper mineralization to occur.[1–3,37] $1,25(OH)_2D$ does have several additional direct effects on osteoblasts by increasing the expression of bone specific alkaline phosphatase, osteocalcin, osteonectin, osteoprotogerin, and a variety of cytokines.[3,36,37] In addition, $1,25(OH)_2D_3$ alters the proliferation and apoptosis of skeletal cells including hypertrophic chondrocytes. These direct actions of $1,25(OH)_2D$ on bone cell homeostasis may explain the severity of rickets in patients with *VDR* mutations.[38] Vitamin D analogs have been developed that have a marked anabolic effect on bone and are being evaluated for the treatment of osteoporosis.[37]

NONCALCEMIC ACTIVITIES OF $1,25(OH)_2D_3$

Most tissues and cells in the body have a VDR, including brain, prostate, breast, gonads, colon, pancreas, heart, monocytes, and activated T and B lymphocytes.[1–6,37] Although the exact physiologic function of $1,25(OH)_2D$ in these tissues is not fully understood, $1,25(OH)_2D$ has varied biological activities that have important physiologic implications and pharmacologic applications. $1,25(OH)_2D_3$, and its analogs inhibit proliferation and induce terminal differentiation of normal cells, such as keratinocytes and cancer cells that express VDR including those of the prostate, colon, breast, lymphoproliferative system, and lung.[1–3,6,37] The antiproliferative and pro-differentiating properties of $1,25(OH)_2D_3$ and its analogs have been successfully developed to treat the hyperproliferative skin disorder psoriasis and are in development to treat prostate, breast, liver, and colon cancer.[5,39–41]

$1,25(OH)_2D$ has been reported to downregulate renin production in the kidney, suggesting that $1,25(OH)_2D$ may influence blood pressure control.[42] β-islet cells have a VDR, and $1,25(OH)_2D_3$ stimulates insulin production and secretion[1–3] either directly through its interaction with the β-islet cell's VDR or indirectly by raising the serum concentration of calcium.

Activated T and B lymphocytes, monocytes, and macrophages all respond to $1,25(OH)_2D$, resulting in the modulation of their immune functions.[1–4,6,37] Thus, it has been suggested that vitamin D sufficiency may be important in decreasing risk of common autoimmune diseases such as multiple sclerosis, Crohn's disease, rheumatoid arthritis, and diabetes type 1[1–4,6,43–45] (Fig. 4).

NONRENAL SYNTHESIS OF $1,25(OH)_2D_3$

The 1-OHase was cloned, and various point mutations have been identified for pseudovitamin D–deficient rickets (vitamin D–dependent rickets type 1).[3,46] In 1-OHase, knockout (KO) mice reproductive and immune dysfunction has been reported.[47] The cloning of the 1-OHase has provided the impetus to explore the expression of this mitochondrial enzyme in nonrenal tissues, including prostate, colon, skin, breast, lung, and osteoblasts.[1–3] Although the physiologic function of the extrarenal 1-OHase is not well understood, there is mounting evidence that the local cellular production of $1,25(OH)_2D$ may be important for regulation of cell growth, immune function, and other cellular activities (Fig. 4). It is believed that once $1,25(OH)_2D$ is made and carries out its physiologic function(s), it induces CYP24 and is rapidly catabolized to calcitroic acid and therefore does not enter into the circulation to increase circulating concentrations of $1,25(OH)_2D$[1,2] (Fig. 1). Further studies are needed to help clarify the importance of the nonrenal 1-OHase in normal and cancer tissues.

REGULATION OF PTH SECRETION BY $1,25(OH)_2D$

The parathyroid chief cell has a VDR and it responds to $1,25(OH)_2D_3$ by decreasing the expression of the *PTH* gene and decreasing PTH synthesis and secretion. Patients with long-standing secondary and tertiary hyperparathyroidism can develop within the parathyroid glands, islands of PTH secreting cells that have little or no VDR. These cells are no longer responsive to the PTH-lowering effect of $1,25(OH)_2D$.[48] Thus, the goal in patients with mild to moderate renal failure is to suppress secondary hyperparathyroidism. This can be accomplished by maintaining normal serum calcium concentrations first by controlling for hyperphosphatemia, which is one of the most potent downregulators of renal production of $1,25(OH)_2D_3$. However, when the serum phosphorus levels are maintained in the normal range and there continues to be an increase in PTH levels in the circulation, the use of oral or intravenous $1,25(OH)_2D_3$ and its less calcemic analogs 19-nor1,25-dihydroxyvitamin D_2, 1α-hydroxyvitamin D_3, 1α-hydroxyvitamin D_2, or 1,24-epi-dihydroxyvitamin D_2, to maintain serum calcium levels and directly suppress PTH expression is warranted.[49] One of the additional benefits of using an active vitamin D analog in chronic renal failure (CRF) patients is to decrease mortality presumably by decreasing cardiovascular events.[50] CRF patients who receive active vitamin D analog still need to be monitored for vitamin D deficiency.[51] It is known that parathyroid glands have 1-OHase activity, and thus increasing 25(OH)D may result in the local production of $1,25(OH)_2D$ in the parathyroid glands, which in turn, could downregulate PTH production.[52]

CLINICAL APPLICATIONS

Hypocalcemic Disorders

There are a variety of hypocalcemic disorders that are directly associated with acquired and inherited disorders in the acquisition of vitamin D, its metabolism to $1,25(OH)_2D$, and the cellular recognition of $1,25(OH)_2D$.[1–3,25,46,53] Vitamin D deficiency can be caused by a decreased synthesis of vitamin D_3 in the skin because of (1) excessive sunscreen use, (2) clothing of all sun-exposed areas, (3) aging, (4) changes in season of the year, and (5) increased latitude.[1–3,53] Intestinal malabsorption of vitamin D associated with fat malabsorption syndromes including Crohn's disease, sprue, Whipple's disease, and hepatic dysfunction are recognizable by low or undetectable circulating concentrations of 25(OH)D.[3,53] Increased vitamin D deposition in body fat is the cause of vitamin D deficiency in obesity.[1,2] Dilantin, phenobarbital, glucocorticoids, and a wide range of other drugs enhance the catabolism of 25(OH)D through the activation of PXR, requiring that these patients receive at least two to five times the AI for vitamin D to correct this abnormality.[22,53] Because the liver has such a large capacity to produce 25(OH)D, usually >90% of the liver has to be dysfunctional before it is incapable of making an adequate quantity of 25(OH)D. Often the fat malabsorption associated with the liver failure is the cause for vitamin D deficiency.[3,53] Patients with nephrotic syndrome excreting >4 g of protein/24 h can have lower 25(OH)D because of the co-excretion of the DBP with its 25(OH)D into the urine.[3,53]

Acquired disorders in the metabolism of 25(OH)D to $1,25(OH)_2D$ can cause hypocalcemia. Patients with chronic renal failure with a glomerular filtration rate (GFR) of <30% of normal have decreased reserved capacity to produce $1,25(OH)_2D$.[1,3,49,53] Hyperphosphatemia and hypoparathyroidism will result in the decreased production of $1,25(OH)_2D$.[3,16,53]

Conversely, hypophosphatemia may be associated with inappropriate low levels of $1,25(OH)_2D$ in the presence of excessive levels of FGF-23 that increases urinary phosphate excretion and inhibits the renal production of $1,25(OH)_2D$.[3,17,54–56] This occurs in patients with abnormally high levels of FGF-23 because of the oncogenic production of the factor and in patients with hereditary activating mutations in the *FGF-23* gene (autosomal dominant hypophosphatemic rickets) and in patients with X-linked hypophosphatemic rickets caused by inactivation mutations in the *PHEX* gene.[53–56] The precise etiology for why $1,25(OH)_2D$ is low in these patients is not well understood.[56]

There are three rare inherited hypocalcemic disorders that are caused by a deficiency in the renal production of $1,25(OH)_2D$: vitamin D–dependent rickets type I, a defect or deficiency in the VDR [$1,25(OH)_2D$-resistant syndrome], and elevated levels of a heterogenous nuclear ribonucleoprotein that specifically interacted with the VDRE, thus preventing the binding of the $1,25(OH)_2D$-VDR-RXR to the VDRE, resulting in resistance to $1,25(OH)_2D_3$ on calcium and bone metabolism.[3,25,46,53,57]

Hypercalcemic Disorders

Excessive ingestion of vitamin D (usually >10,000 IU/day) for many months can cause vitamin D intoxication that is recognized by markedly elevated levels of 25(OH)D (usually >150 ng/ml), and normal levels of $1,25(OH)_2D$, hypercalcemia, and hyperphosphatemia.[3,53,58] Ingestion of excessive quantities of $25(OH)D_3$, 1α-OH-D_3, $1,25(OH)_2D_3$, dihydrotachysterol, and other active vitamin D analogs or exuberant use of topical $1,25(OH)_2D_3$, calcipotriene (Dovonex; Leo Pharmaceutical Corp.), and other active vitamin D analogs for psoriasis can cause vitamin D intoxication.[5,53,58,72] Because activated macrophages convert 25(OH)D to $1,25(OH)_2D$ in an unregulated fashion, chronic granulomatous diseases such as sarcoidosis and tuberculosis are often associated with increased serum levels of $1,25(OH)_2D$ that results in hypercalciuria and hypercalcemia.[3,15,53] Rarely, lymphomas associated with hypercalcemia are caused by increased production of $1,25(OH)_2D$ by macrophages associated with the lymphoma. Primary hyperparathyroidism and hypophosphatemia are also associated with increase renal production of $1,25(OH)_2D$.[3,16,49,53]

CONSEQUENCES AND TREATMENT OF VITAMIN D DEFICIENCY

Vitamin D plays a critical role in the mineralization of the skeleton at all ages. As the body depletes its stores of vitamin D because of lack of exposure to sunlight or a deficiency of vitamin D in the diet, the efficiency of intestinal calcium absorption decreases, causing a decrease in the serum ionized calcium concentrations, which signals the calcium sensor in the parathyroid glands to increase the synthesis and secretion of PTH.[1–3,49,53] PTH not only conserves calcium by increasing renal tubular reabsorption of calcium, but mobilizes stem cells to become active bone calcium resorbing osteoclasts. PTH also increases tubular excretion of phosphorus, causing hypophosphatemia. The net effect of vitamin D insufficiency and vitamin D deficiency is a normal serum calcium, elevated PTH and alkaline phosphatase, and low or low normal fasting serum phosphorus. The hallmark of vitamin D deficiency is a low level of 25(OH)D (<15 ng/ml) in the blood.[1–3,53,59] Most experts agree that to minimize PTH levels the 25(OH)D in adults should be >30 ng/ml.[1–3,59–62] The secondary hyperparathyroidism and low calcium-phosphorus product is thought to be responsible for the detective mineralization action of the

osteoid, which is the hallmark of rickets/osteomalacia.[1,3,53,63] In addition, the secondary hyperparathyroidism causes increased osteoclastic activity, resulting in calcium wasting from the bone, which in turn can precipitate or exacerbate osteoporosis.

Vitamin D deficiency is a major cause of metabolic bone disease in older adults. Rickets is, once again, becoming a major health problem for infants of mothers of color who exclusively breast feed their children and do not supplement them with vitamin D.[1,53,63,64] Infants fed only nondairy milk substitutes that contain neither calcium nor vitamin D are also prone to developing rickets.[65] Vitamin D deficiency is underappreciated in both children and adults (1–3,12,66–69). The NHANES III survey revealed that 42% of black women 15–49 years of age were vitamin D deficient throughout the United States at the end of the winter.[67] Vitamin D deficiency is common in the elderly,[1,12,66] especially those who are infirm and in nursing homes.[1–3,12,53,66] Approximately 50% of free-living elders in Boston and Baltimore were found to be vitamin D deficient throughout the year.[1,53,66] Even healthy children and young adults are at risk. Forty-eight percent of preadolescent white girls (9–11 years of age)[66] and 52% of Hispanic and black adolescent boys and girls were reported to have a 25(OH)D of <20 ng/ml in New England.[69] In Europe where vitamin D fortification is scarce, 78% of Finnish girls (10–12 years of age) were vitamin D deficient.[70] In sunny climates where children and adults avoid sun exposure including Lebanon, Saudi Arabia, and India, a high incidence of rickets is reported.[66,71,72] A study in students and young medical doctors 18–29 years of age in Boston at the end of the winter revealed 32% were vitamin D deficient, and a significant number had secondary hyperparathyroidism.[66] Vitamin D deficiency not only robs the skeleton of precious calcium stores but causes osteomalacia. This disease, unlike osteoporosis, causes vague symptoms of bone pain, bone achiness, muscle aches and pains, and muscle weakness. These symptoms are often dismissed by physicians, and the patient is given the diagnosis of fibromyalgia, chronic fatigue syndrome, or simple depression.[66] Eighty-eight percent of Arab women with muscle pain, weakness, and bone pain[66] and 93% of children and adults 10–65 years of age with similar complaints were found to be vitamin D deficient.[73]

Casual exposure to sunlight is the best source of vitamin D. Because the skin has such a large capacity to produce vitamin D_3, children and adults of all ages can obtain their vitamin D requirement from exposure to sunlight. For young adults, a whole body exposure to one minimum erythemal dose (a slight pinkness to the skin) of simulated sunlight was found to be equivalent to taking a single oral dose of between 10,000 and 25,000 IU of vitamin D.[1,2] Therefore, children and adults only need minimum exposure of unprotected skin to sunlight followed by the application of a sunscreen or use of other sun protective measures, including clothing. In Boston, it is recommended that white men and women expose face and arms or arms and legs to sunlight three times a week for ~25% of time that it would cause a mild sunburn in the spring, summer, and fall.[1–3,66]

Patients with vitamin D deficiency require immediate attention with aggressive therapy. Trying to replete vitamin D deficiency with a multivitamin or with a few multivitamins containing 400 IU of vitamin D is not only not effective, but can be potentially dangerous because one multivitamin often contains the safe upper limit of vitamin A. The vitamin D tank is empty and requires rapid filling. This can be accomplished by giving pharmacologic doses of vitamin D orally; 50,000 IU of vitamin D_2 (equal to ~15,000 IU of vitamin D_3) once a week for 8 weeks will often correct vitamin D deficiency as measured by an increase in 25(OH)D >20 ng/ml[68] (Fig. 5). If this is not achieved, an additional 8-week course is reasonable. For patients who are chronically vitamin D deficient, they are often given 50,000 IU of vitamin D_2 once or twice a month after correction of their vitamin D deficiency. The fortification of some orange juices and other juice products with vitamin D[9] offers an additional source of vitamin D to those who do not drink milk. An effective alternative, especially for patients with malabsorption syndrome, is to be exposed to UVB radiation. In a nursing home setting, the installation of UVB-emitting lamps in an activity room was more effective then a multivitamin in maintaining 25(OH)D levels.[1,66] A patient with Crohn's disease, severe vitamin D deficiency osteomalacia, and attendant bone pain was effectively treated by being exposed to a sunbed three times a week.[1,66] The goal is to have blood levels of 25(OH)D of at least 30 ng/ml to achieve the maximum benefit for skeletal and cellular health.[1,10,11,61] For children with rickets, a single oral dose of 200,000 IU of vitamin D_3 is often effective in correcting the vitamin D deficiency.[63]

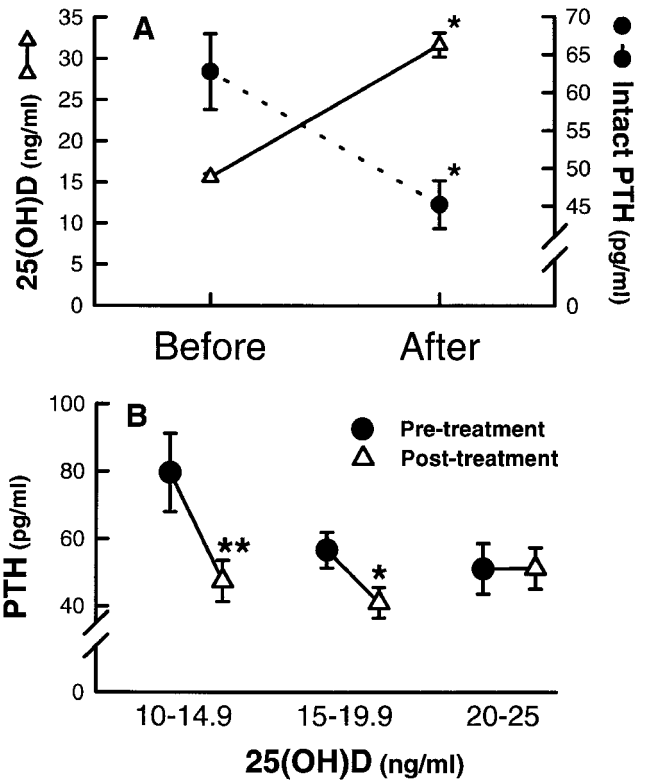

FIG. 5. (Top) Healthy adults 49–83 years of age received 50,000 IU of vitamin D_2 once a week for 8 weeks. Serum levels of 25(OH)D and intact PTH are shown before and after receiving vitamin D_2. (Bottom) These same patients with serum 25(OH)D >10 (considered to be the lowest limit of the normal range by many laboratories) and <25 ng/ml were stratified regarding their PTH levels before and immediately after therapy. The data clearly show significant declines in PTH when the initial serum 25(OH)D were between 11 and 19 ng/ml, suggesting that patients were deficient in vitamin D until the 25(OH)D was >20 ng/ml. Based on this and other data, the new recommendation is that patients should have a blood level of at least 20 ng/ml and preferably 30 ng/ml of 25(OH)D to maximize bone and cellular health.

CONCLUSION

When evaluating patients for hypo- and hypercalcemic conditions, it is appropriate to consider the patient's vitamin D

status as well as whether they suffer from either an acquired or inherited disorder in the acquisition and/or metabolism of vitamin D. Because the assay for vitamin D is not available to clinicians, the best assay to determine vitamin D status is 25(OH)D. The $1,25(OH)_2D$ assay is not only useless in determining vitamin D status, but it can also be misleading because $1,25(OH)_2D$ levels can be normal or elevated in a vitamin D–deficient patient. Only when there is a suspicion that there is an acquired or inherited disorder in the metabolism of 25(OH)D is it reasonable to measure circulating $1,25(OH)_2D$ concentrations. Although there are a variety of other metabolites of vitamin D in the circulation, the measurement of other vitamin D metabolites has not proved to be of any significant value. It has been suggested that there may be a correlation with the development of metabolic bone disease with the polymorphism for the *VDR* gene. Although these data are intriguing, the information is, at this time, of limited clinical value but may someday provide an insight as to a person's potential maximum BMD. The noncalcemic actions of $1,25(OH)_2D_3$ have great promise for clinical applications in the future (Fig. 4). The activated vitamin D compounds $1,25(OH)_2D_3$, 22-oxo-$1,25(OH)_2D_3$, $1,24(OH)_2D_3$, and calcipotriene herald a new pharmacologic approach for treating psoriasis.[5] The recent report of a vitamin D analog that markedly increased BMD in rodents offers a novel approach of developing vitamin D analogs as anabolic drugs to treat osteoporosis.[37] High-dose vitamin D_3 therapy or combination therapy of $1,25(OH)_2D_3$ and its analogs with other cancer drugs offers promise in treating many lethal cancers.[39,41,66] In addition, vitamin D analogs are being developed to treat or mitigate common autoimmune disorders including multiple sclerosis and diabetes type 1, as well as hypertension, cardiovascular heart disease, and prostatic hypertrophy. Clearly the vitamin D field of research remains robust.

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Chapter 18. Calcitonin

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INTRODUCTION

Calcitonin (CT) is a 32 amino acid peptide secreted by thyroidal C cells and acting to inhibit osteoclasts in their resorptive function. This property has led to its use for disorders characterized by increased osteoclastic bone resorption, such as Paget's disease, osteoporosis, and the hypercalcemia of malignancy. The secretion of CT is regulated acutely by blood calcium and chronically by sex and perhaps age. Calcitonin is metabolized by the kidney and the liver. Calcitonin is also a tumor marker for medullary thyroid carcinoma, the signal tumor of multiple endocrine neoplasia (MEN) type II.[1–4]

BIOCHEMISTRY

Over a dozen species of CT, including human, have been sequenced. Common features include a 1–7 amino-terminal disulfide bridge, a glycine at residue 28, and a carboxy-terminal proline amide residue.[2–4] Five of the nine amino-terminal residues are identical in all CT species. The greatest divergence resides in the interior 27 amino acids. Basic amino acid substitutions enhance potency. Thus, the nonmammalian CTs have the most potency, even in mammalian systems. A biologically active fragment of CT has not been discovered.[5–7]

MOLECULAR BIOLOGY

The *CT* gene consists of six exons separated by introns.[2,4] Two distinct mature mRNAs are generated from differential splicing of the exon regions in the initial gene transcript. One translates as a 141-residue CT precursor and the other as a 128-residue precursor for calcitonin gene-related peptide (CGRP). CT is the major post-translationally processed peptide in C cells, whereas CGRP, a 37 amino acid peptide, is the major processed peptide in neurons. The main biological effect of CGRP is vasodilation, but it also functions as a neurotransmitter and does react with the CT receptor. The relevance of CGRP to skeletal metabolism is unknown, but it may be produced locally in skeletal tissue and exert a local regulatory effect. An alternative splicing pathway for the *CT* gene produces a carboxy-terminal C-pro CT with eight different terminal amino acids.[1–3]

BIOSYNTHESIS

Thyroidal C cells are the primary source of CT in mammals, and the ultimobranchial gland is the primary source in submammals.[1,3] C cells are neural crest derivatives, and they also produce CGRP, the second *CT* gene product. Other tissue sources of CT have been described, notably the pituitary cells and widely distributed neuroendocrine cells.[2,4] Although CT may have paracrine effects at these sites, the nonthyroidal sources of CT are not likely to contribute to its peripheral concentration. However, malignant transformation can occur in both ectopic and eutopic cells that produce CT, and the peptide becomes a tumor marker.[1] The best example of the latter is medullary thyroid carcinoma, and of the former, small-cell lung cancer.[2,8]

The author has reported no conflicts of interest.

BIOLOGIC EFFECTS

CT's main biological effect is to inhibit osteoclastic bone resorption.[1] Within minutes of its administration, CT causes the osteoclast to shrink in size and to decrease its bone-resorbing activity.[2] This dramatic and complex event is accompanied by the production of cAMP and by increased cytosolic calcium in the osteoclast.[2,3] In a situation where bone turnover is sufficiently high, CT will produce hypocalcemia and hypophosphatemia. Calcitonin has also been reported to inhibit osteocytes and stimulate osteoblasts, but these effects are controversial.[2,6] Analgesia is a commonly reported effect of CT treatment.[3,4] Calciuria, phosphaturia, and gastrointestinal effects on calcium flux have been reported for CT, but they occur at concentrations of the hormone that are supraphysiologic.[1–3] It should be noted, however, that the concentration of the peptide at its several sites of biosynthesis may be sufficiently high to explain some extraskeletal effects of CT by a paracrine mechanism.[1] Thus, CT may exert physiologic effects on the pituitary and CNS.[1,7] Furthermore, the demonstration of CT and CT receptors at intracranial sites may qualify CT as a neurotransmitter.[1,8] Other effects of CT have been reported. It has been observed to act as an anti-inflammatory agent, to promote fracture and wound healing, to be uricosuric, to be antihypertensive, and to impair glucose tolerance. The importance of these latter effects is yet to be determined.[1–4]

CALCITONIN AS A DRUG

CT's main biological action of inhibiting osteoclastic bone resorption has resulted in its successful use in disease states characterized by increased bone resorption and the consequent hypercalcemia.[2,3] Calcitonin is widely used in Paget's disease, in which osteoclastic bone resorption is dramatically increased; in osteoporosis, in which the increase of bone resorption may be more subtle; and in the treatment of hypercalcemia of malignancy.[2,5] Newer pharmacologic preparations of CT may have improved therapeutic effects.[2,4]

SECRETION AND METABOLISM

Ambient calcium concentration is the most important regulator of CT secretion.[1] When blood calcium rises acutely, there is a proportional increase in CT secretion, and an acute decrease in blood calcium produces a corresponding decrease in plasma CT.[1,2] The C cells seem to respond to sustained hypercalcemia by increasing CT secretion, but, if the hypercalcemia is severe and/or prolonged, the C cells probably exhaust their secretory reserve. Chronic hypocalcemia seems to decrease the secretory challenge to C cells and they increase their stores of CT; these stores can be released on appropriate stimulation.[2,4,9] The metabolism of CT is a complex process that involves many organ systems. Evidence has been reported for degradation of the hormone by kidney, liver, bone, and even the thyroid gland.[2–4] Like many other peptide hormones, CT disappears from plasma in a multiexponential manner that includes an early half-life measured in minutes. Inactivation of the hormone seems more important than renal excretion, because relatively little CT can be detected in urine. Metabolism of the peptide during inflammatory disease may result in the

production of specific forms of CT that can be identified by selective immunoassays.[1]

GASTROINTESTINAL FACTORS

Gastrointestinal peptides, especially those of the gastrin-cholecystokinin family, are potent CT secretagogues when administered parenterally in supraphysiologic concentrations.[1,2,9] This observation has led to the postulate that there is an entero-C-cell regulatory pathway for CT secretion. However, only meals that contain sufficient calcium to raise the blood calcium have been shown to increase CT secretion in humans.[4] Nevertheless, pentagasrtin as well as glucagon can be used as stimulatory test for CT secretion.[2,9]

PROVOCATIVE TESTING FOR CT-PRODUCING TUMORS

The stimulatory effect of calcium and gastrointestinal (GI) peptides, especially pentagastrin, on CT secretion has led to their use as provocative tests for the secretion of CT in patients suspected of having medullary thyroid carcinoma (MTC), a neoplastic disorder of thyroidal C cells that can occur in a familial pattern as part of MEN type II.[1,9] Most tumors respond with increased CT secretion to the administration of either calcium or pentagastrin or their combination, but either agent can sometimes give misleading results. CT measurements can also be used to monitor the effectiveness of therapy, usually thyroidectomy, in patients with MTC.[1–3]

SEX AND AGE

Most investigators find that women have lower CT levels than men.[1,2,4] The effect of age on CT secretion is more controversial[1,2]: newborns seem to have a higher serum level of the hormone, and in adults, a progressive decline with age has been reported by several but not all laboratories.[1,4] The physiologic significance of the various circulating forms of CT measured by different assay procedures has not been defined.

MEDULLARY THYROID CARCINOMA

Medullary thyroid carcinoma is a tumor of the CT-producing C cells of the thyroid gland.[1,3,5] Although a rare tumor, it can occur in a familial pattern as part of MEN type II. Medullary thyroid carcinoma is generally regarded as intermediate between the aggressive behavior of anaplastic thyroid carcinoma and the more indolent behavior of papillary and follicular thyroid carcinoma. These tumors usually produce diagnostically elevated serum concentrations of CT. Therefore, immunoassay for CT in serum can be used to diagnose the presence of MTC with an exceptional degree of accuracy and specificity. To identify these patients with early disease, provocative tests for CT secretion have been developed.[2,9]

OTHER CT-PRODUCING NEOPLASMS

Neoplastic disorders of other neuroendocrine cells can also produce abnormally elevated amounts of CT. The best known example is small-cell lung cancer. However, other tumors, such as carcinoids and islet cell tumors of the pancreas, can also overexpress CT.[1,2,4]

RENAL DISEASE

There are increases in immunoassayable CT with both acute and chronic renal failure, but considerable disagreement exists regarding the mechanism and significance of these increases.[2,3] Because the secretion and/or metabolism of CT is abnormal in renal disease, and because renal osteodystrophy is characterized by increased bone resorption, CT, which acts to inhibit bone resorption, has been implicated in the pathogenesis of uremic osteodystrophy.[1,2,4]

HYPERCALCIURIA

Elevated levels of CT have been shown in patients with hypercalciuria.[4,10] The physiologic significance of enhanced CT secretion is unknown, but it may represent a compensatory response to intestinal hyperabsorption of calcium.

BONE DISEASE

No skeletal disease has been conclusively attributed to CT abnormalities.[1,3,6] Although women have lower CT levels than men, there is conflicting evidence as to whether endogenous secretion of the hormone contributes to the pathogenesis of osteoporosis.[1] Nevertheless, CT has been of therapeutic benefit in osteoporosis.[3,4] Reduced CT reserve in women may contribute to the greater severity of osteitis fibrosa cystica in women with primary hyperparathyroidism.[1] Skeletal abnormalities have not been identified in patients after thyroidectomy.[1,2] However, the recent demonstration in pycnodysostosis of dysfunctional mutations of the gene for cathepsin K, responsible for collagen degradation by osteoclasts, and the high levels of serum calcitonin reported in this disease, suggest an intriguing link between skeletal dysplasia and calcitonin secretion.[1,4] For example, the increased calcitonin may be compensatory for the impaired osteoclastic resorption.

HYPERCALCEMIA AND HYPOCALCEMIA

Calcium challenge is a well-documented stimulus for CT secretion. Although increased CT secretion has only inconsistently been associated with chronic hypercalcemia, an exaggerated response of CT to secretagogues has been convincingly observed in several hypocalcemic states.[4,9]

CALCITONIN RECEPTOR

CT mediates its biological effects through the CT receptor (CRT).[4,8] CTRs are most robustly expressed in osteoclasts but also are expressed in several other sites, including the CNS. The mammalian CTRs share common structural and functional motifs, signal through several pathways, and can exist in several isoforms with insert sequences or deletions or both in their intracellular and extracellular domains. These isoforms arise from alternative splicing of receptor mRNA transcribed from a single gene. Some of the isoforms of the CTR seem to have differential ligand specificity, perhaps accounting for the pleiotropic effects of the hormone. Salmon calcitonin (SCT) apparently conforms best to the structural requirements for binding and signaling of mammalian CTRs, perhaps explaining its greater potency in humans and mammals and its sustained receptor binding and activation of cAMP.[1,2] An isoform of the CTR that is expressed in rat brain seems to preferentially recognize SCT, and binding sites for SCT are present at several sites in the CNS. It is thus speculated that an SCT-like ligand is produced by mammals. There is some evidence for this in both humans and murine. Finally, it is also notable that another nonmammalian CT, chicken CT (CCT), also seems more potent in humans than human CT and that a CCT-like ligand may be expressed in humans.[2,4]

LIGAND FAMILIES FOR CALCITONIN

Long known to be related to CGRP1 and 2, CT recently was recognized to be related in sequence to two other peptides,

adrenomedullin and amylin.[1,8] Although all of these peptides share the feature of being neuromodulators, they also seem to have unique hormone actions. The effects of CT were described earlier. CGRP1 and CGRP2 are potent vasodilators an immunomodulators, with actions in the CNS and at many other targets. Adenomedullin also is a potent vasodilator with some CNS actions. The actions of amylin are related to carbohydrate metabolism, to gastric emptying, and to CNS function.[4,8]

RECEPTOR MODULATION

Despite their distinct bioactivities, the family of calcitonin-related ligands shows some cross-reactivity at each other's receptors, although they generally bear only partial homology.[2,4,8] The interaction of ligands among these receptors is influenced by newly discovered receptor-modulating proteins.[8] This modulation expands the repertoire of biological actions that can be mediated by receptors and their ligands. One modulator, termed CGRP-receptor component protein (CGRP-RCP), consists of a hydrophilic protein that is highly conserved across species and found in virtually all tissues. The second group of receptor modulators is a family of proteins termed receptor-activity-modifying proteins (RAMPs) and comprises three members, RAMPs1 to 3.[2,4,8] RAMPs interact with a distinct calcitonin receptor-like receptor (CRLR) and transports it to the cell-membrane surface.[4,8] RAMP1 transport of CRLR results in a terminally glycosylated receptor that recognizes CGRP, whereas RAMP2 and 3 expression produces a core glycosylated receptor that recognizes adrenomedullin. Receptor modulation is another molecular mechanism of genetic economy whereby specific ligands can acquire functions beyond those allowed by a simple lock-and-key model of receptor specificity.[8]

ROLE OF CALCITONIN IN MINERAL METABOLISM

The exact physiologic role of CT in calcium homeostasis and skeletal metabolism has not been established in humans, and many questions remain unanswered about the significance of this hormone in humans. Does CT secretion decline with age? Do gonadal steroids regulate the secretion of CT? Do the lower levels of serum CT in women contribute to the pathogenesis of age-related loss of bone mass and osteoporosis? Do extrathyroidal sources of CT participate in the regulation of skeletal metabolism? Are there primary and secondary abnormalities of CT secretion in diseases of skeletal and calcium homeostasis? The conclusive answers to these questions await clinical studies with an assay procedure that directly measures the biological activity of CT in blood. Furthermore, accurate local measurements of CT and its effects may be necessary to elucidate the emerging role of CT as a paracrine and autocrine agent.

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