

Hormonal Control of Calcium Homeostasis

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Calcium homeostasis in the extracellular fluid is tightly controlled and defended physiologically. Hypercalcemia always represents considerable underlying pathology and occurs when the hormonal control of calcium homeostasis is overwhelmed. The major hormones that are responsible for normal calcium homeostasis are parathyroid hormone and 1,25-dihydroxyvitamin D; these hormones control extracellular fluid calcium on a chronic basis. Over- or underproduction of these hormones or the tumor peptide, parathyroid hormone-related peptide, are the major causes of aberrant extracellular fluid calcium concentrations. These hormonal defense mechanisms are reviewed here.

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Control of Calcium Homeostasis

The extracellular fluid (or plasma) calcium concentration is tightly controlled by a complex homeostatic mechanism involving fluxes of calcium between the extracellular fluid (ECF)¹ and the kidney, bone, and gut. These fluxes are carefully regulated by three major hormones: parathyroid hormone (PTH), calcitonin, and 1,25-dihydroxyvitamin D [1,25(OH)₂D₃]. Important cellular functions are dependent on the maintenance of the extracellular calcium concentration within a narrow range (1). Disturbances of this tightly regulated homeostatic system leads to disorders of calcium metabolism that have predictable effects, which can be ascribed to effects on these cellular functions.

The approximate fluxes of calcium into and out of the ECF that occur during each 24-h period are shown in Fig. 1. Usually, bone mineral accretion equals skeletal mineral resorption, and calcium content in the urine approximates that of net intestinal absorption. An average Western diet provides a calcium intake of ~1 g of elemental calcium per day. Typically, ~30% (300 mg) is absorbed, the majority across the small intestine and a small percentage in the colon (2, 3). Because gut secretion of calcium is

relatively constant at 150 mg per day, the net calcium absorption is ~150 mg per day for a healthy adult in normal calcium balance. Calcium absorbed from the gut enters the blood and is filtered by the kidney. The majority of filtered calcium (>98%) is reabsorbed in the proximal renal tubules; thus, only 150 mg per day is excreted in healthy individuals (4).

The skeleton is the major body storage site for calcium. A healthy adult contains ~1–1.3 kg of calcium, and 99% of this is in the form of hydroxyapatite in the skeleton (5). The remaining 1% is contained in the ECF and soft tissues. Additionally, <1% of the skeletal content of calcium is in bone fluid and exchanges freely with the ECF.

Although the hormonal control of calcium fluxes is central to understanding of normal calcium homeostasis, Parfitt and co-workers (6–11) have also emphasized the importance of physico-chemical exchanges of calcium between the bone fluid and the ECF. The bone fluid is rich in calcium because it is in equilibrium with the mineral phase of bone at the bone surface. The exchanges between the bone fluid and the ECF may be important in determining the set point (mean concentration of serum calcium at steady state) and error correction (by which serum calcium is returned to the set point and corrected by oscillations in the ionized calcium concentrations about this mean). The relative importance of this exchange mechanism has been underappreciated.

Neuman showed almost 40 years ago that there is a special bone fluid that is analogous to the cerebrospinal fluid. This bone fluid is separated from the ECF by a "bone membrane", which is probably composed of the bone lining cells that cover bone surfaces in a continuum (10, 12–14). This bone membrane functions to keep calcium in the ECF and out of the bone (the ECF is supersaturated with calcium compared with both the bone fluid and the crystalline surface of bone). The hormonal mechanisms that might control calcium fluxes across the bone membrane are unknown at present, as are any possible influences on these fluxes by disease states such as the

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¹ Nonstandard abbreviations: ECF, extracellular fluid; PTH, parathyroid hormone; 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D; and PTH-rP, PTH-related protein.

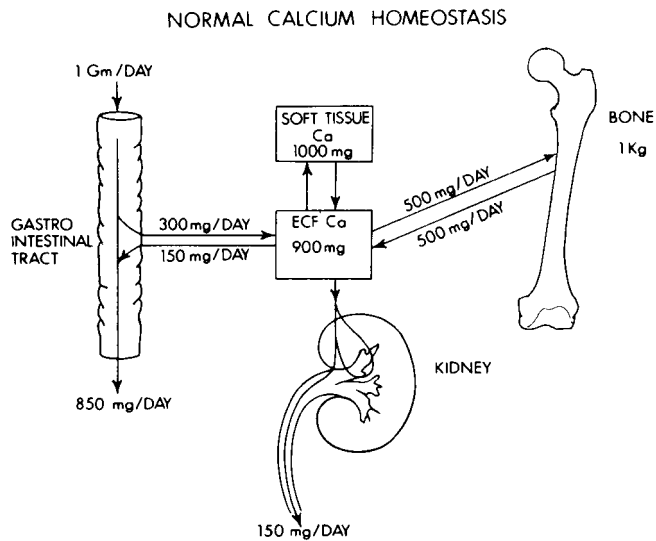


Fig. 1. Calcium homeostasis in a healthy adult over 24 h.

hypercalcemia of malignancy or primary hyperparathyroidism. However, it is possible that these fluxes buffer fluctuations in ECF calcium caused by, for example, dietary calcium loads or calcium entry from bone destruction caused by malignancy. For example, these fluxes may be important in determining set point. They could also be important in returning plasma calcium to the steeping (error correction) after a calcium load.

Hormonal Effects on Calcium Homeostasis

Blood ionized calcium concentrations are remarkably stable in healthy individuals because of the homeostatic system involving the actions of the three calciotropic hormones on the target organs of bone, gut, and kidney, and possibly also on fluxes between the bone canalicular fluid and the ECF mentioned above. Normal calcium homeostasis is primarily dependent on the interactions of PTH, $1,25(\text{OH})_2\text{D}_3$, and calcitonin on these organs to maintain the ionized calcium concentration within a very narrow range. Other factors also influence calcium fluxes, although current evidence suggests that only these three hormones are under negative feedback control.

PTH AND PTH-RELATED PEPTIDE

PTH is an 84-amino acid peptide that is synthesized by the chief cells of the parathyroid gland. Secretion of PTH is highly dependent on the ionized calcium concentration and represents a simple negative feedback loop. The serum PTH concentration decreases as the serum calcium concentration increases, although PTH secretion is not entirely suppressible (15). However, there is a relatively narrow range of regulation of PTH secretion by extracellular calcium, with little further effect when total corrected serum calcium is >2.9 mmol/L (11.5 mg/dL) or <2.1 mmol/L (8.5 mg/dL). The calcium-sensing receptor that mediates this negative feedback has recently been cloned from bovine parathyroid cells (16). This G-protein-

linked receptor is mutated in the disorders of familial hypocalciuric hypercalcemia (17), neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia (18, 19). Synthesis of PTH is likely maximal under normal physiologic conditions because parathyroid cells exposed to hypercalcemic conditions in vitro display a decrease in mRNA for PTH, whereas those exposed to hypocalcemic conditions do not show such an increase (20). Active vitamin D metabolites decrease PTH synthesis in vitro and in vivo (21, 22) as well.

The biological actions of PTH include (a) stimulation of osteoclastic bone resorption and release of calcium and phosphate from bone, (b) stimulation of calcium reabsorption and inhibition of phosphate reabsorption from the renal tubules, and (c) stimulation of renal production of $1,25(\text{OH})_2\text{D}_3$, which increases intestinal absorption of calcium and phosphate. The amino-terminal end of the PTH molecule binds to the PTH receptor to elicit these biologic responses. The PTH receptor has recently been cloned and found to be a member of the large family of receptors that contain a seven transmembrane-spanning domain and work through activation of G-proteins (23).

PTH metabolism is complex and produces several fragments of varying biological and immunological reactivity. The intact and biologically active peptide has a half-life in the circulation of <4 min (24). Intact PTH is cleared rapidly by kidney and liver (25–27). Hepatic Kupffer cells take up intact PTH and degrade it into very small peptides as well as cleave it into discrete fragments that are released into the circulation (28–30). The released carboxy-terminal fragments circulate considerably longer than the intact hormone, mainly because they are cleared exclusively by glomerular filtration (28, 31, 32). The complex metabolism and circulating heterogeneity of PTH are likely reasons for the difficulty encountered in developing good PTH assays. Highly sensitive and specific immunoradiometric assays for intact PTH are now widely available (33).

The tumor peptide PTH-related protein (PTH-rP) was first discovered with in vitro bioassays for PTH in tumors derived from lung, breast, and kidney (34–36). This factor is now known to be expressed by many squamous cell carcinomas and has also been described in T-cell lymphomas that present with humoral hypercalcemia (37). It is a 141-amino acid peptide and shares considerable homology with PTH in the first 13 amino acids. It binds to and activates the PTH receptor, and this is presumably the reason it mimics the biological effects of PTH on bone, kidney, and the gut. It stimulates osteoclastic bone resorption and promotes renal tubular calcium reabsorption in similar concentrations to that of native PTH (38). In some models of hypercalcemia associated with increased PTH-rP, hypercalcemia can be reversed by passive inoculation with neutralizing antibodies to PTH-rP (39). It is now known that PTH-rP is produced by $\sim 50\%$ of primary breast cancers and its production may be enhanced at the bone site by bone-derived factors such as transforming

growth factor- β (TGF β) (32, 40, 41). RIAs have been developed for PTH-rP, although these assays have not shown a perfect relationship between the presence and severity of hypercalcemia and expression of the protein (35, 42, 43).

It is now clear that PTH-rP has a pathophysiological role not just in hypercalcemia but also in local osteolysis. Immunohistochemistry has been used to demonstrate that there is increased expression of PTH-rP in bone sites compared with either soft tissue metastases or primary tumors in patients with carcinoma of the breast (32). This has been shown experimentally by inoculation of the human breast cancer cell line MDA-MB-231 into the left cardiac ventricles of nude mice. Osteolytic lesions caused by metastasis occur over the following 4–6 weeks, and there is an increase in PTH-rP expression in the tumor cells that metastasize to bone. When tumor-bearing nude mice are treated with neutralizing antibodies to PTH-rP, not only is there a decrease in the development of the osteolytic bone lesions, but there is also a decrease in the tumor burden in bone (40).

The physiological role of the PTH-rP remains unclear. It probably has no regulatory effect on calcium homeostasis under physiological conditions. It is produced in healthy skin cells as well as in amniotic cells, and it may have effects on epithelial cell replication and on smooth muscle contraction during labor (44). It is also expressed by lactating breast tissue and is present in large amounts in breast milk. However, most recent interest has focused on its potential local effects in cartilage differentiation. PTH-rP knockout experiments performed by introducing the null-mutation into the germ line of mice have shown that the mice have died before birth of an abnormality of the rib cage that causes impaired respiration (45). These abnormalities are caused by an enhanced cartilage cell differentiation and normal ossification in the rib cage. Thus, PTH-rP is a naturally occurring and essential inhibitor of cartilage cell differentiation, and its absence leads to abnormalities at the growth plate. Overexpression experiments using transgenic mice in which PTH-rP expression is targeted to cartilage cells by use of the type II collagen promoter show a marked delay in endochondral ossification (46) and also demonstrate cartilage abnormalities (46). Its effects on cartilage cells seem to be mediated by Indian hedgehog protein produced by prehypertrophic cartilage cells in the growth plate (47, 48).

Two PTH receptors have been identified. The more recently described receptor is a G-protein-coupled receptor that shares 51% amino acid homology with the well-known PTH/PTH-rP receptor, but PTH appears to be the major and possibly only active ligand (49–51). The importance of this second PTH receptor is not clear, and there are many important questions that need to be addressed. These include the effects of PTH-rP on this receptor and whether these are identical to those of PTH, whether this receptor can explain some of the controversial non-bone effects of PTH that have been described for

many years such as those on the vascular system, what the signal transduction pathway that is connected to this receptor is, and finally whether this receptor is related to the anabolic response of PTH. Early studies suggest the receptor is not as responsive to PTH-rP as it is to PTH and that it may mediate its effects through cAMP and intracellular calcium signal transduction pathways (51).

CALCITONIN

Calcitonin is a 32-amino acid peptide that is synthesized and secreted by the parafollicular cells of the thyroid gland. The ionized calcium concentration is the most important regulator of calcitonin secretion (52). Increases in ionized calcium produce an increase in calcitonin secretion, and conversely, a fall in the ambient calcium concentration inhibits calcitonin secretion. Gastrointestinal peptide hormones, gastrin in particular, are potent calcitonin secretagogues. This likely is responsible for increased calcitonin secretion after meals, but the physiologic relevance of this observation remains unclear. Pentagastrin, a gastrin analog, is used as a provocative stimulus to determine the capacity of a patient to secrete calcitonin (53).

The precise biological role of calcitonin in the overall schema of calcium homeostasis is uncertain. Calcitonin directly inhibits osteoclastic bone resorption (54), and the effect is rapid, occurring within minutes of administration. This inhibition is accompanied by the production of cAMP (55) as well as an increase in cytosolic calcium (56) in the osteoclast and leads to contraction of the osteoclast cell membrane (57). These effects are transient and likely have little role in calcium homeostasis chronically, although they may be important in short-term control of calcium loads. Clinical observations support the notion that calcitonin has little chronic effect because neither calcitonin-deficient patients (athyroid) nor patients with medullary thyroid cancer and excess calcitonin production experience alterations in calcium homeostasis. The calcitonin receptor has been cloned (58) and is structurally similar to the PTH receptor in that it also has seven transmembrane domains. Calcitonin is metabolized in minutes in the circulation, predominantly in the kidney (1). The calcitonin receptor is related structurally to the PTH/PTH-rP and secretin receptors (58). The calcitonin receptor exists in several isoforms, and its expression seems to be influenced by ambient concentrations of calcitonin itself. This may be the reason for down-regulation of the receptor and the escape phenomenon that occurs in the continued presence of calcitonin (59).

VITAMIN D METABOLITES

The steroid hormone 1,25(OH)₂D₃ is the major biologically active metabolite of the vitamin D sterol family. The vitamin D precursor (previtamin D₃) is either ingested in the diet or synthesized in the skin from 7-dehydrocholesterol through exposure to sunlight (60). Hydroxylation occurs in the liver at the C-25 position to form 25-

hydroxyvitamin D, the substrate for the more potent metabolite, $1,25(\text{OH})_2\text{D}_3$. 25-Hydroxyvitamin D is hydroxylated at the C-1 position in the kidney by 1α -hydroxylase, a complex cytochrome P_{450} mitochondrial enzyme system located in the proximal nephron (61), to form $1,25(\text{OH})_2\text{D}_3$ (62–64). The renal 1α -hydroxylation of 25-hydroxyvitamin D is the major recognized control point in vitamin D metabolism, responding to ambient phosphate concentrations, circulating PTH concentrations, and calcium concentrations. PTH and phosphate depletion act independently to increase $1,25(\text{OH})_2\text{D}_3$ production, PTH being the more potent stimulus. $1,25(\text{OH})_2\text{D}_3$ also acts via its receptor to inhibit renal 1α -hydroxylase activity. Nephrectomy abolishes the 1α -hydroxylase activity. The only other known important extrarenal sites of $1,25(\text{OH})_2\text{D}_3$ production are in the placenta and granulomatous tissue (65–67). The half-life of $1,25(\text{OH})_2\text{D}_3$ in the circulation is ~ 5 h in humans. Fifteen percent is excreted as urinary metabolites and 50% as fecal metabolites (4).

$1,25(\text{OH})_2\text{D}_3$ increases plasma calcium and phosphate concentrations by increasing the absorption of calcium and phosphate from the gastrointestinal tract (64). It also increases bone resorption (68) and enhances the effects of PTH in the nephron to promote renal tubular calcium reabsorption. It is a powerful differentiation agent for committed osteoclast precursors (69,70), causing their maturation to form multinucleated cells that are capable of resorbing bone. By these actions, $1,25(\text{OH})_2\text{D}_3$ provides a supply of calcium and phosphate available at bone surfaces for the formation of normal mineralized bone.

Whether 24,25-dihydroxyvitamin D_3 has effects on bone metabolism has been a controversial issue for years. Many people have believed it to be biologically inert. However, recent studies in null-mutant mice in which the 25-hydroxyvitamin D 24-hydroxylase gene is deleted throw doubt on this notion (71). Although the heterozygotes are apparently normal, approximately one-half of the homozygotes die before weaning, apparently because of hypercalcemia associated with nephrocalcinosis. Although the bone histology is normal in the first generation of homozygotes, the second generation show an accumulation of osteoid tissue at sites of intramembranous ossification, such as in the calvaria, clavicle, mandible, and periosteal surface of long bones. These studies suggest that 24,25-dihydroxyvitamin D_3 may play an important role in normal intramembranous bone formation.

Recently, there have been further clarifications of the receptor mechanisms and response elements in target genes for 1,25-dihydroxyvitamin D. The gene that has been used most frequently for studying transcriptional effects of the vitamin D receptor is the osteocalcin gene, which has provided a wealth of information. It is apparent that the vitamin D receptor functions as a transcription factor (like other members of the steroid hormone superfamily) and causes effects on target genes by forming a heterodimer with the retinoic acid receptor, and this

Table 1. Defenses against hypocalcemia and hypercalcemia.

Protection against decreased plasma calcium (e.g., caused by dietary or hormonal deficiency)
Glomerular filtration: filtered load of calcium decreases
Calcitropic hormones (PTH, $1,25(\text{OH})_2\text{D}_3$, and calcitonin)
Effects on bone and kidney: hypocalcemia stimulates PTH release, which increases plasma calcium by effects on renal tubules and osteoclasts
Effects on gut (adaptation): increased fractional absorption of dietary calcium, mediated by $1,25(\text{OH})_2\text{D}_3$
Protection against increases in plasma calcium (caused by bone destruction or large dietary calcium load)
Glomerular filtration: filtered load of calcium increases
Calcitropic hormones
PTH; but no further decrease in secretion if plasma calcium >2.9 mmol/L (11.5 mg/dL)
Calcitonin; but no long-term efficacy
$1,25(\text{OH})_2\text{D}_3$; but gut effects are slow and limited

is responsible for the mediation of its effects on gene expression (72).

Defenses against Hypercalcemia and Hypocalcemia

The usual physiologic defenses against hypercalcemia and hypocalcemia are listed in Table 1. The majority of these defense mechanisms are mediated through the hormonal actions of PTH and $1,25(\text{OH})_2\text{D}_3$.

A fall in ionized calcium concentration is immediately sensed by the parathyroid glands, which respond with an increase in PTH secretion. PTH increases osteoclastic bone resorption, releasing calcium and phosphate from bone into the ECF. PTH also causes increased renal tubular reabsorption of calcium as well as inhibition of phosphate reabsorption. PTH stimulates synthesis of $1,25(\text{OH})_2\text{D}_3$, which further increases absorption of calcium and phosphate from the gut. If these mechanisms are intact, the extracellular calcium concentrations should return to normal.

In the converse situation, a rise in ionized calcium concentration causes a decrease in PTH secretion from the parathyroid glands. Thus, renal tubular calcium reabsorption and osteoclastic bone resorption are decreased. Synthesis of $1,25(\text{OH})_2\text{D}_3$ is also decreased, which in turn decreases absorption of dietary calcium and phosphate. Thus, a healthy individual responds to increases in ionized calcium with an increase in renal calcium excretion and a decrease in intestinal absorption of calcium.

In general, these hormonal responses are more effective in protecting against hypocalcemia than hypercalcemia. Perturbations in these mechanisms as exemplified by excessive increases in bone resorption, deficiencies or excesses of PTH or $1,25(\text{OH})_2\text{D}_3$, and defects in renal capacity to handle calcium and phosphate will lead to either hypercalcemia or hypocalcemia.

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