Evidence for an important physiological role for calcitonin
(calcium storage/bone fluid calcium homeostasis/postprandial calcium)

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ABSTRACT We propose that calcitonin, secreted in response to the intake of food, aids in routing calcium, obtained by intestinal absorption, into bone fluid. Here calcium is temporarily stored in combination with phosphate for return to the extracellular fluid (blood) during intervals between oral intakes of calcium. The net result is a conservation of calcium postprandially and a decrease in parathyroid hormone-induced bone destruction during subsequent fasting periods. Evidence for this postulate is provided in the following six sequential steps from the time a calcium-containing meal is consumed until that portion of calcium stored in bone fluid is utilized during fasting periods to aid in plasma calcium maintenance. (i) Calcitonin secretion is stimulated by feeding and subsequent digestive processes. (ii) Postprandial secretion of calcitonin restricts the efflux of calcium from bone fluid to blood, thereby maintaining parathyroid hormone (PTH) secretion. (iii) In thyroid-intact individuals, both PTH and calcitonin are secreted postprandially and act in concert on calcium homeostasis. (iv) Calcitonin actively moves phosphate into bone and prevents its loss from bone fluid to blood. (v) Postprandial storage of calcium with phosphate occurs in bone fluid of thyroid-intact individuals. (vi) This labile storage form of calcium is the first to be utilized during fasting periods. In the absence of partial disruption of this storage mechanism, rapid development of pathological bone conditions would not be expected because PTH action permits the extended utilization of bone calcium for plasma calcium control. However, augmentation of osteopenic conditions could be expected if long-term low calcium intake were accompanied by a malfunction of this calcitonin-induced system for calcium storage.

The existence of calcitonin was first reported by Copp et al. (1) in 1962. In the following year it was established by Hirsch et al. (2) as a hypocalcemic principle, extractable from thyroid tissue. In spite of the fact that calcitonin has been found in plasma samples taken from all mammals studied (including man), its basic physiological role has remained in doubt. It has often been labeled as a "hormone in search of a function." There is, as yet, no indication that its absence from man or other mammals is life-threatening. However, it is difficult to believe that a hormone known to circulate in blood of so many different animals does not have a demonstrable role.

Calcitonin is known to be both a hypocalcemic and a hypophosphatemic agent, and at the time of its discovery it was considered to be an important regulator of plasma calcium concentration (1, 2). Because plasma calcium levels remain in the normal range in the absence of any calcitonin-secreting tissue, this role was changed to one that characterized it as an antihypocalcemic hormone (3, 4). This function was proposed because endogenous calcitonin secretion will prevent or decrease the hypercalcemia that follows calcium administration by intravenous infusion, intraperitoneal injection, or gavage (5, 6). Although calcitonin acts as an antihypocalcemic agent in these nonphysiologic conditions, it is very difficult to raise the plasma calcium concentration above normal, even in thyroidecomized animals, by feeding a meal containing a high content of calcium (7, 8). In addition, hypercalcemic conditions in man due to hyperparathyroidism, tumors, or other internal disorders are not prevented by the presence of calcitonin-secreting tissue.

The most thoroughly tested action of calcitonin has been its ability to suppress osteoclastic-induced bone resorption (9, 10). The osteoclast's ruffled border, the metabolically active area in bone resorption, is decreased in both its amount and its activity after calcitonin administration (11). This action of calcitonin is considered to be the primary reason for its therapeutic effectiveness in the treatment of Paget's disease.

Recent studies have demonstrated that endogenous calcitonin decreases the efflux of calcium from fluid bathing bone surfaces to the primary extracellular fluid compartment (12, 13). This action is central in the development of the following report in which we present evidence for what we hypothesize to be an important, and possibly the primary, physiological role for calcitonin. We propose that this function of calcitonin is of physiological significance in man as well as in other mammals because supporting evidence has also been obtained from clinical studies.

SYNOPSIS OF PROPOSED PHYSIOLOGICAL ROLE FOR CALCITONIN

We propose that calcitonin, secreted in response to food entering the digestive tract, stimulates rapid storage of a portion of the absorbed calcium in bone fluid in a readily available form. This storage is a separate, distinct process from that of the more stable adsorption of calcium on bone surfaces. The calcium stored in bone fluid is present in a labile (metastable) form which is kept from being transformed to hydroxyapatite crystals by the presence of a mineralization inhibitor. Upon cessation of calcium absorption from the gut and the decrease in circulating calcitonin levels, the stored calcium is gradually returned to the extracellular fluid. This process decreases postprandial calcium loss in the urine and supplies calcium to maintain plasma concentrations during fasting periods. The net result permits a decrease of parathyroid hormone (PTH) secretion during fasting periods so that its bone resorptive activity can be minimized. After extended periods of time, any deficiency or failure in this storage process could aggravate conditions of osteopenia caused by low dietary calcium intake.

The suggested site for calcium storage in bone fluid is illustrated in a diagram in Fig. 1 and in electron micrographs of the shaft of the tibia fixed in lanthanum in Fig. 2. Lanthanum replaces calcium, thereby slowing deposition of the colloid. Fig. 3 provides calcium "flow" diagrams for both thyroid-intact and thyroidecomized individuals to illustrate the differences that

Abbreviation: PTH, parathyroid hormone.
FIG. 1. Diagram of the organs and tissues involved in the proposed mechanism for calcitonin-induced storage of calcium. Boxed portion at left is a slightly magnified area of bone surface to demonstrate the site of storage of calcium in bone fluid.

FIG. 2. Transmission electron micrographs of sections from the diaphyseal area of a rat tibia after fixation in lanthanum nitrate. The electron-dense precipitate demonstrates the proposed sites of a calcitonin-induced calcium storage in bone fluid. (A) Lanthanum precipitate (arrow) is in the bone fluid area between bone surface (B) and the bone-lining cells (Lc). (X6700.) (B) Pericellular lanthanum localization (arrow) is within the lacuna of an osteocyte (Oc). (X7000.)
1. Calcitonin Secretion Is Stimulated by Feeding and Subsequent Digestive Processes. There is no doubt that endogenous calcitonin secretion can be stimulated by an increase in plasma calcium levels above normal (14, 15). There is recent evidence that, in the rat, plasma calcitonin levels are increased after feeding (8, 16, 17). This has also been reported to occur in man but, because of conflicting evidence, cannot be considered to be established (18). It is important to point out that, in rats conditioned to a specific feeding schedule, plasma calcium concentrations decrease slightly during the feeding process before an increase in plasma calcitonin can be identified (16, 19). The situation is different in man, in whom it has been reported that calcitonin secretion or injection during the feeding process actually prevented rather than caused a postprandial decrease in plasma calcium concentrations (20). These studies suggest that factors other than calcium may be important in stimulating calcitonin release.

Care et al. (21), Cooper and his collaborators (22–25), and Garel (26) have clearly shown that intestinal hormones may act as secretagogues for calcitonin. Although gastrin is suspected to be a secretagogue in man, an as-yet-unidentified principle is believed to be the secretagogue in rats, in which gastrin is not effective in inducing calcitonin secretion. Our contribution to this effort includes a collaborative study with Cooper (16) demonstrating that, in rats maintained on a regulated feeding schedule, plasma calcitonin levels increased rapidly after feeding. This occurred at a time when plasma calcium levels were at their lowest. It appears that the necessity for an increase in plasma calcium levels as a prerequisite for the postprandial secretion of calcitonin is in doubt, but the cause of this increased secretion of the hormone remains to be clarified.

The absence of calcitonin-secreting tissue in the rat results in an easily identifiable increase in postprandial renal calcium excretion. This can be returned to normal by a single low-dose injection of calcitonin postprandially (27). From studies in both man and the rat we concluded that, in thyroidectomized individuals, postprandial renal calcium excretion was primarily a reflection of the amount of calcium ingested during the immediate past meal. However, in thyroid-intact individuals, the amount of calcium in the urine was influenced by the amount of calcium consumed on the preceding day (27, 28).

These studies implicate digestive processes in the secretion of calcitonin. They also suggest that, in the presence of calcitonin, the calcium obtained from the diet is utilized for maintenance of plasma calcium beyond the duration of the biological life of the hormone in a manner significantly different from that in thyroidectomized subjects.

2. Postprandial Secretion of Calcitonin Restricts the Efflux of Calcium from Bone Fluid to Blood, So That PTH Secretion Is Not Completely Shut Off by Movement of Calcium from the Intestines into Blood. If calcitonin is secreted after food consumption, then we can assume that its subsequent actions include some of those produced by the administration of calcitonin. Although it is recognized that calcitonin, especially in large amounts, is able to inhibit bone resorative processes (9, 11), our data have led us to conclude that the primary effect of a lower, more physiological, dose of calcitonin is to decrease the efflux of calcium from bone fluid to blood (12, 13). Experimentally, this would produce a hypocalcemia, leading in turn to a decrease in calcium influx into bone fluid because the influx is not metabolically controlled (29). If the decrease of calcium influx from bone fluid occurred simultaneously with the entrance of calcium from the intestines into blood, a hypocalcemia would not necessarily result. Therefore, postprandial calcitonin secretion could produce a decrease in calcium efflux from bone fluid without causing a decrease in plasma calcium concentra-tion (27). Under such conditions, the net result of postprandial calcitonin secretion would be to maintain the rate of calcium influx into bone fluid at nearly normal rates.

The secretion of PTH is known to be controlled by a negative feedback mechanism with ionic calcium (30), so that one might expect the influx of dietary calcium into the blood to inhibit PTH secretion. However, inhibition of PTH secretion would be prevented or minimized by the action of postprandial calcitonin secretion which decreases calcium efflux from bone fluid with or without detectable changes in plasma calcium levels. According to this reasoning, both PTH and calcitonin would be secreted postprandially in thyroid-intact individuals after they consumed a calcium-containing meal. In contrast, after the same meal is consumed by thyroidectomized individuals, PTH secretion would be decreased or completely lacking. Direct evidence for higher postprandial plasma PTH levels in thyroid-intact than in thyroidectomized humans or experimental animals is yet to be obtained. However, indirect evidence in support of this concept has been provided in a study of postprandial urinary calcium changes in the rat (27). One of the established actions of PTH is to increase renal tubular reabsorption of calcium (31), and our results show that postprandial renal calcium excretion after a calcium-containing meal is much lower in thyroid-intact than in thyroidectomized rats.

3. In Thyroid-Intact Individuals, Both PTH and Calcitonin Are Secreted Postprandially and Act in Concert on Calcium Homeostasis. Although not all possible advantages or disadvantages of the simultaneous secretion of these supposedly "antagonistic" hormones are known, one important advantage can be defined. It is well established that PTH increases osteoclastic bone resorption (32, 33). However, at low doses and independently of osteoclastic activity, PTH also rapidly increases the rate of calcium efflux from bone fluid to blood (34–36). At the surfaces of bone, the two hormones (PTH and calcitonin) have antagonistic effects on the calcium efflux system. When injected or secreted, calcitonin temporarily overrides the action of PTH (13), causing a suppression of calcium efflux (bone fluid to blood) even in the presence of PTH. In contrast, calcitonin does not override the renal actions of PTH. In the presence of both hormones, the ability of PTH to increase renal tubular reabsorption of calcium is undiminished (37). Therefore, a net result of simultaneous postprandial secretion of both hormones is a decrease in the renal loss of calcium absorbed from the diet. At the same time, despite the action of calcitonin to temporarily override the effect of PTH on calcium efflux from bone fluid to blood, calcium influx into bone fluid is unaffected, and calcium supplies obtained by intestinal absorption are directed into bone fluid.

4. Calcitonin Actively Moves Phosphate into Bone and Also Prevents Its Loss from Bone Fluid When an Ion Gradient Is Created by a Decrease in Plasma Phosphate Concentrations. PTH and calcitonin are both hypophosphatemic, and the decrease in plasma phosphate levels is augmented by simultaneous injection of both hormones. The hypophosphatemia that follows PTH administration is a direct result of the phosphaturia produced by this hormone. In fact, recent studies (38, 39) suggest that the plasma phosphate decrease would be even greater if it were not partially compensated for by an entry of phosphate into blood from extravascular sources, due to the shift in the ion gradient produced by the hypophosphatemia.

Calcitonin, on the other hand, causes hypophosphatemia by moving phosphate out of blood. This concept has received its support primarily from work (40–42) carried out in our laboratories with 32P and 45Ca as tracers. The interpretation of the
data was based on specific effects of PTH and calcitonin on plasma radioisotope specific activities. Discussion of experimental tracer procedures is beyond the scope of this report, but results of these studies (37–39) permitted the conclusion that bone rather than soft tissue was the most probable site of deposition of the phosphate leaving plasma after calcitonin injection.

A recent study (37) utilizing $^{32}$P as a tracer demonstrated that, when administered with PTH, calcitonin augmented the decrease in plasma phosphate but did not either increase or decrease the PTH-induced phosphaturia. The ability of calcitonin to override the PTH-induced changes in plasma $^{32}$P specific activity allowed us to conclude that calcitonin prevented the loss of phosphate from bone fluid, which occurred when PTH was given alone, thereby holding phosphate in bone fluid.

Our studies on effects of these two hormones on plasma phosphate concentrations have led to the conclusion that one of the results of postprandial calcitonin secretion is the conservation of bone fluid phosphate. The two hormones acting in concert postprandially direct the new supplies of calcium absorbed from the intestines into bone fluid, which at that time is maintained maximally with phosphate.

5. Storage of Calcium with Phosphate Occurs in Bone Fluid or Near the Surfaces of Bone As the Result of the Calcitonin-Induced Postprandial Sequence of Events. Evidence has accumulated for the formation of a calcium phosphate colloid in bone fluid postprandially when calcitonin-secreting tissue is present. In order for this to occur, bone lining cells and osteocytes near bone surfaces must respond rapidly to both calcitonin and PTH. Morphological changes in these cells were first reported by Matthews et al. (43) and have been confirmed in a series of reports (44–46). Sufficient evidence now exists to convince us that these actions of the cells that control calcium fluxes between bone fluid and blood and are the cells that furnish the metabolic stimulus for the postprandial storage of calcium with phosphate in bone fluid.

The first evidence that calcitonin injection could cause the formation of calcium phosphate compounds in bone fluid was provided by Matthews et al. (43). Under the special conditions of their experiment this material transformed to hydroxyapatite, establishing that it contained both calcium and phosphate. Recently, by the use of various electron micrographic procedures using stains known to react with calcium or phosphate, we have been able to identify an electron-dense material in bone fluid after calcitonin injection. These procedures include fixation of rat tibia specimens with lanthanum or potassium pyroantimonate, post-fixation with lead, and the use of anhydrous procedures (47). Finally, in experiments not yet completed, we have been able to identify an electron-dense material in bone fluid of tissue samples removed from thyroid-intact rats 4 hr after they consumed a calcium-containing meal. This material was present in smaller quantities or was absent in similar preparations from thyroidectomized rats.

We believe that these studies provide evidence for the conclusion that, due to the action of calcitonin and PTH on the bone lining cell/osteocyte unit, bone fluid responds to the entrance of calcium provided postprandially by the formation of a calcium phosphate colloid. This colloid does not transform into hydroxyapatite and thus is readily available for transfer back into blood.

6. This Labile Storage Form of Calcium Is the First Calcium to be Utilized During Fasting Periods, Thereby Decreasing the Amount of PTH Required to Maintain Plasma Calcium Levels. One function of this proposed calcium storage system may be to provide calcium for plasma calcium maintenance during intervals between oral intakes of calcium.

Without such a supply, additional PTH secretion would be required, calling on bone calcium efflux mechanisms and bone resorptive processes for the needed calcium.

The evidence that this stored calcium is returned to blood during fasting periods is indirect. It is based on clinical studies and physiological experiments rather than on the bone fluid loss of an identifiable material—studies not yet done. For example, in a preliminary study, Grubb et al. (28) found that postprandial urinary calcium values in thyroidectomized patients were correlated positively with the amount of calcium consumed in the most recent meal. In contrast, these values in age-matched thyroid-intact controls were not correlated with the calcium in this meal but instead had a positive correlation with the amount of calcium consumed during the previous day.

In our recent studies in rats, we found that the pattern of postprandial urinary calcium excretion was established by the presence of calcitonin-secreting tissue or its replacement by postprandial administration of calcitonin to thyroidectomized rats (27). Thus, the situation in rats is similar to that reported for man. An important point made in this study was that the effects of a single postprandial injection of calcitonin in thyroidectomized rats provided with a calcium-containing meal permitted these rats to respond identically to thyroid-intact rats on the second day when neither calcium nor calcitonin was provided.

If this calcitonin-induced system for postprandial storage of calcium (which is released during fasting) operates as predicted, one of the major effects should be on the daily cycle of PTH secretion. This should be higher in thyroid-intact than in thyroidectomized individuals postprandially, when only its renal actions are manifested. However, during fasting periods, when neither dietary calcium nor calcitonin is available, PTH secretion should be lower in thyroid-intact than in thyroidectomized subjects.

ROLE OF THIS STORAGE SYSTEM IN HEALTH AND DISEASE

The question immediately arises as to whether the presence or absence of such a hormone-induced calcium storage system plays a necessary role in normal physiology or in the etiology of pathological conditions. Our evidence for its existence in man as well as in the laboratory rat is substantial. On the other hand, removal of calcitonin-secreting tissue does not appear to produce immediate harmful effects in either species. The ability of PTH to modulate the calcium efflux system from bone fluid to blood and to increase the rate of bone resorption as an additional source of calcium is such that plasma calcium concentrations can be maintained within normal limits. Any effect of a malfunction in this system therefore must require considerable time for development of a recognizable disorder.

The standard diet normally provided the laboratory rat is abnormally high in its calcium content (1.2% dry weight). On this diet, a 200-g rat has a daily intake of calcium (225 mg) equal to that of many human subjects. It is quite possible that, due to this high calcium intake, the rat maintained in the laboratory would not show specific effects from the lack of calcitonin and the calcium storage system.

The condition in humans is somewhat different. The average daily requirement for calcium is officially listed at 800 mg. In addition, public health statistics indicate that the average person in the United States has a daily intake considerably below the recommended amount. It is our belief that malfunction of this calcium storage system in humans on a low-calcium intake could augment or produce osteopenic conditions. It is also our hope that detection of such malfunction and its correction by postprandial administration of calcitonin might aid the individual in fully utilizing his or her daily calcium intake.
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