

Editorial

Calcitonin, an enigmatic hormone: does it have a function?

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Abstract

This editorial presents our view of the status of thyroidal calcitonin (TCT) in mammalian physiology. The discovery of calcitonin (CT) enabled the development of a valuable therapeutic agent but the early experiments most likely misled us with regard to its physiological significance. These early purported roles for TCT, first as an agent important in blood calcium regulation and later as an agent to prevent hypercalcemia, are no longer considered as physiological functions. While large supraphysiological doses of CT have an effect on the morphology and function of osteoclasts, it is unlikely that these effects of CT are important in the normal physiology of osteoclasts or bone remodeling. It is surprising that 38 years after the discovery of TCT there is no consensus as to its role in normal mammalian physiology. This editorial concerns three possibilities with respect to TCT: 1) the hormone is vestigial; 2) the hormone plays a role in water metabolism, ionic concentrations, and/or acid-base balance, actions that may not involve calcium metabolism at all; and 3) TCT acts to store phosphate postprandially on bone surfaces as a labile calcium-phosphate colloid, an action that may provide calcium needed for use in non-feeding periods or to reduce postprandial loss of phosphate when dietary phosphate is limited. Also discussed are recent publications indicating that CT synthesized in non-thyroidal tissues (NTCT) may have paracrine actions.

Keywords: Thyroidal Calcitonin, Calcitonin Functions, Calcium-Phosphate, Bone Surfaces, Water and Ion Balance

Introduction

Historically, three hormones are considered to be important physiologically in the regulation of calcium homeostasis in mammals: parathyroid hormone (PTH), vitamin D, and calcitonin (CT). As emphasized in our previous editorial in this journal, PTH is recognized as the most important agent responsible for the maintenance of blood calcium¹. Also imperative for calcium homeostasis is vitamin D; it has been proven to be essential for intestinal calcium transport as well as calcium movement through bone cells². On the other hand, vitamin D has never been considered to have a direct physiological role in the hour to hour control of a constant plasma calcium concentration. When thyroidal CT (TCT) was discovered over 35 years ago, most investigators believed that TCT was a hypocalcemic hormone important for the regulation of blood calcium by opposing PTH. However, the experiments leading to the discovery of TCT may have misled us. So far, no physiological

role for TCT has been established, in part because a deficiency syndrome has not been identified. Thus, it is possible that one or more of the following hypotheses should be considered: 1) TCT is a vestigial hormone, or 2) TCT may affect pH and/or the excretion of water and ions under stressful conditions, functions that do not involve calcium metabolism at all; and 3) TCT may function to form on bone a readily reversible calcium-phosphate colloid from ingested calcium that becomes available during periods of deprivation. This editorial will examine these possibilities for TCT. Recent studies suggest important paracrine involvement of CT synthesized in non-thyroidal tissues with implantation and mammary gland development. Because of these findings, it seems proper to discuss the function of TCT separate from that of non-thyroidal CT (NTCT).

Why is TCT considered a calcium - regulating hormone?

The discovery of TCT occurred as the result of two different types of experiments, neither of which was performed under physiological conditions. Both may have inadvertently led investigators to the conclusion that the

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hormone was of primary importance in calcium metabolism. The first, by Copp et al.³, involved perfusing blood very high in calcium content (30% above normal levels) through the thyroid-parathyroid complex isolated from dogs. When the perfusate was returned to dogs, their blood calcium fell. Because the fall in blood calcium was more rapid than that observed after thyroparathyroidectomy, Copp et al. concluded that the hypocalcemia produced by the perfusate was due to a humoral substance. Initially, the hypocalcemic agent, named calcitonin, was thought to be of parathyroid origin³.

Experiments by Hirsch et al.⁴ compared the fall in serum calcium levels in rats parathyroidectomized by hot-wire cautery to that in rats parathyroidectomized by surgical excision of the gland. Cautery produced a more rapid and a more profound fall in serum calcium, which led to the conclusion that hot-wire treatment released a hypocalcemic agent from the thyroid gland. Extracts of thyroid glands from rats, as well as from other species, were shown to lower serum calcium of both intact and surgically parathyroidectomized rats⁴. These experiments helped to establish the thyroid gland as the source of the hypocalcemic agent and to support the concept that TCT was a calcium-regulating hormone. The sequence of events leading to the discovery of TCT, the early experiments, the confirmatory studies, and suggested functions for the hormone that include evidence for a calcium-related mechanism were reviewed in detail by Hirsch and Munson⁵.

Now, 38 years after its discovery, none of the proposed functions of TCT has been clearly established. And no TCT deficiency or excess syndromes have been identified. Although large amounts of TCT are secreted in patients with medullary thyroid carcinoma, no biological effect, such as hypocalcemia, occurs⁶. Nevertheless, there are good reasons to believe that TCT may be of importance in calcium metabolism. Foremost is the presence of calcium receptors on the C-cells that regulate the secretion of TCT. These receptors are similar in structure to the overall family of hormonal receptors⁷. Thus, increasing blood levels of calcium stimulate the secretion of TCT. Most endocrine glands respond to increased levels of serum calcium by increasing hormonal secretion but this response usually occurs only in the presence of an activator⁸. Examples of activators are ACTH and TSH for the secretion of glucocorticoids and thyroxine, respectively. In contrast, TCT and PTH do not appear to require activators⁸. While there are known secretagogues for TCT (for example, gastrin in pigs and humans, but not in rats), the actions of these agents appear to be species specific and related to eating. The importance of these secretagogues is not known. Although TCT is secreted from the thyroid gland and circulates at picomolar concentrations in human blood, its function has not been elucidated.

The effects of administering large doses (micromolar) of exogenous CT to young animals^{9,10} should not be considered as representative of the actions of TCT in normal physiology. In general, care should be exercised not to ascribe

physiological functions to substances that are administered at supraphysiological doses. For example, high doses of glucocorticoids are used therapeutically and effectively as immunosuppressive and anti-inflammatory agents. However, one would be reluctant to ascribe primary physiological significance to these effects seen at supraphysiological doses. It is also important to realize that exogenous CT does not lower serum calcium in adult humans. Medically, exogenous CT lowers calcium in hypercalcemic patients when hypercalcemia is due to excessive bone resorption^{10,11}. Clearly, there are pharmacological effects of CT. However, evidence for a physiological role of TCT is still lacking.

Possible functions for TCT and NTCT

As suggested in the Introduction, this editorial emphasizes three possibilities for TCT: a vestigial hormone; an important factor in ion-pH-water regulation under stress; and an agent for the sequestration of consumed calcium phosphate as a reversible colloid complex on bone to be available during deprivation. However, other functions are also likely and should be considered. In this regard, it is important to emphasize that TCT is secreted by cells that originate in the neural crest and that TCT as well as the calcitonin gene-related peptide (CGRP), the other peptide synthesized by alternate processing of the CT gene, may have a role in neural function or neural transmission. Recognition of receptor location is also helpful to reveal possible functions. Receptors for CT, or a CT-like compound, have been found in the central nervous system. Furthermore, exogenous CT has been shown to have analgesic properties, although at high concentrations. Receptors have also been identified in the kidney, gastrointestinal tract, and heart. There are suggestions that CT has anti-inflammatory properties and serves to prevent deposition of calcium in the cardiovascular system¹⁰. Other suggested functions include roles in growth, pregnancy, lactation^{6,12}, and vitamin D metabolism¹³. A detailed discussion of the actions and possible functions of endogenous CT has been published by Azria¹⁴. While many possible roles for TCT have been suggested, we feel that one or all of the hypotheses presented in this editorial may be correct.

Very important for this discussion is the finding that CT is synthesized in several non-thyroidal tissues. Twenty years ago Silva and Becker¹⁵ found NTCT in 25 different human tissues; they obtained evidence that CT was synthesized in lung tissue. More recently Zhu et al.¹⁶ showed that attenuation of CT gene expression in the pregnant rat uterus decreased embryonic implantation. Support for a role of NTCT in reproduction was also obtained by McDonald et al.¹⁷ who found reduced litter size in homozygous CT gene-deleted female mice. Also, Tverberg et al. observed that CT-gene expression and CT-receptors were induced in rat mammary tissue between days 12 and 19 of pregnancy¹⁸. Their results point to a proliferative function of CT on the mammary gland. These studies imply paracrine functions for NTCT in

the uterus and mammary gland, and very likely in other tissues as well. Much more work is needed to establish the functions and mechanisms of action of endogenously secreted non-thyroidal CT.

Having presented other functions for TCT and a paracrine role for non-thyroidal CT, we will now elaborate our three hypotheses.

Hypothesis 1. TCT is a vestigial hormone

As there are no apparent deficiency syndromes in humans or experimental animals, the possibility exists that TCT is a vestigial hormone^{6,12}. Although CT is a highly conserved hormone whose existence dates back to the earliest vertebrates, evidence for TCT as an important regulator of calcium metabolism in normal vertebrate physiology is lacking. The theory that TCT deficiency leads to bone loss observed in thyroidectomized humans has been discounted; currently, the bone deficiency observed is thought to be due to inappropriate replacement therapy with classical thyroid hormones¹⁹.

Perhaps the gene has survived because of the need to synthesize, by alternate splicing, CGRP. This peptide is most commonly found in neural synapses. However, unfortunately, the function of CGRP, like TCT, is still not known. Early evidence obtained from the CT gene-knockout mouse has not revealed substantial evidence regarding its function on bone or in neural tissue. Although older knockout mice had less bone²⁰ – a result consistent with the ascribed actions of TCT in calcium metabolism – more recent work from the same laboratory²¹ indicated that CT knockout mice have more bone mass than normal controls, a result not consistent with the putative role of TCT.

That TCT is purely a vestigial hormone seems unlikely for the following reasons. 1) The primary peptide structure has been highly conserved during evolution. Although amino acid sequences differ substantially between species, all vertebrate CTs have 32 amino acids, a 1-7 disulfide ring at the amino terminus and a proline amide at the carboxy terminus. This constancy of structure between species suggests, but does not prove, that function has also been maintained. 2) The blood levels of TCT are approximately the same as other hormones with known actions. 3) Receptors for calcium that induce secretion of CT have been identified on C-cells. Clearly, changes in calcium levels regulate the secretion of TCT²². 4) TCT has significant effects on bone lining cells (see Hypothesis 3, below). 5) During evolution the C-cells migrated from the ultimobranchial body into the thyroid gland of mammals. The possibility that this migration has important physiological significance must be considered since, among studies examining possible thyroid interactions, iodine deficiency was found to induce hypercalcemia in rats^{23,24}.

Taken together, it seems unlikely that a hormone, phylogenetically older than PTH²⁵, would retain structure, maintain secretory regulation, and be present in blood without functional significance. It is more likely that dietary

and environmental conditions under which we, as well as experimental animals, exist are not conducive to observing the effects of CT-deficiency (see Hypotheses 2 and 3, below) and that emphasis on function has been focused on incorrect systems. It is reasonable to propose that the physiological function of CT may not involve calcium, a concept that has led to the development of Hypothesis 2 described below. It is also possible that the main physiological functions of CT have changed over evolutionary time by exaptation - to such effects as the paracrine and other functions described in the section on "Possible functions". The latter interpretation is consistent with the observation that salmon CT (sCT) is substantially more effective in humans than human CT (hCT) and that there is a substantial difference in the amino acid sequences of sCT and hCT.

Hypothesis 2. TCT regulates water, ion concentrations and/or acid-base balance

This hypothesis proposes that TCT is important in the control of water and electrolyte as well as acid-base balance during stressful situations to prevent dehydration. This assumption emphasizes the possibility that the function of TCT may be similar to CT in marine fish and mammals and not directly related to calcium metabolism. It is regrettable that the precise physiology of CT in marine fish has not been elucidated. Such information about CT, a phylogenetically old hormone originating in early vertebrates, could be very helpful in predicting an evolutionary role for TCT in mammals.

Important physiological mechanisms exist in marine fish to prevent dehydration, as the osmotic concentration of seawater exceeds that in extracellular fluid. This regulation of ion and water transport is a major function of the kidney, gills, and the rectal gland²⁶. The close proximity of the gills to the ultimobranchial gland suggests that the secreted CT may be a major factor in regulating water and ion movements. This connection is supported by experiments on gills of salmon by Milhaud et al.^{27,28}. They perfused isolated gills from pre-spawning (seawater) and post-spawning (fresh water) salmon and found that CT, in addition to decreasing calcium and phosphate influx, also decreased the rate of flow of the perfusate²⁷. In a subsequent paper²⁸, using a different technique with isolated gills of salmon, Milhaud et al. reported that CT decreased the rate of flow of the perfusate while the efflux of both calcium and phosphate increased. Unfortunately, fluxes of ions other than calcium and phosphate were not measured. While their emphasis was on the effect of CT to diminish net flux of calcium and phosphate to protect the "milieu interieur", the results provided substantial evidence that CT has profound effects on fluid flow.

Kenny²⁹, aware that CT lowered blood calcium in some but not all lower vertebrates, made the following statement at a meeting in Chapel Hill in 1977: "What, then, is calcitonin's role in lower vertebrates? Again I have no answer. Perhaps CT is playing a physiological role in lower vertebrates different from its mammalian action of inhibiting

bone resorption, and we are looking at the wrong parameter". At the same meeting Copp et al.³⁰ commented that the location of the ultimobranchial glands was anatomically close to the gills of fish, where ion regulation occurs. Also, at the same meeting, Robertson concluded that, after ultimobranchialectomy in frogs, the buffering capacity was altered and the animals developed metabolic acidosis³¹. Later, Robertson also found that frogs, maintained in fresh water following ultimobranchialectomy, developed transitory diuresis³². Despite these early suggestions that the function of CT may not be related to calcium metabolism, major research efforts on lower vertebrates, just as in mammals, were oriented to find effects of CT on calcium metabolism and bone. Such endeavors seem unwise in hindsight since the physiology of lower vertebrates differs so markedly from that of mammals. In marine fish, there is no need to conserve calcium as the concentration of calcium in seawater exceeds that found in blood. Also, C-cells, the source of CT, exist in cartilaginous fish. Unlike humans, fish do not have parathyroid glands to regulate blood calcium but have an ichthyic hormone, stanniocalcin, which acts as a calcium-regulating hormone³³. These, and other factors that are markedly different in lower compared to higher vertebrates, should have led investigators to seek other functions for CT.

Mammals, like marine fish, must have mechanisms to prevent dehydration. Many of the mechanisms are known and act to minimize loss of water during periods of deprivation. The suggestion that a major function for CT in fish may involve prevention of dehydration may also apply to mammals. Mammals, like man and experimental animals, usually have a water supply sufficient to prevent dehydration. However, mammals in desert environments and/or humans with severe water restriction or diarrhea may require CT to assist in the regulation of water, electrolyte, and acid base balance. In support of this hypothesis are the findings that primary targets for TCT are bone and kidney. With regard to the function of bone, Frost³⁴ emphasized the importance of bone in ion regulation. He states, "Both electrolyte and acid-base substances exchange between the blood and the bone tissue. The mineral content of the bone tissue provides a large sink-and-reservoir that, by the way of blood flow through bone, allows the body's homeostatic mechanisms to meet otherwise potentially lethal challenges". That TCT might influence acid-base metabolism via the skeletal system should be considered. The initial action could involve changes in phosphate as proposed in Hypothesis 3, below. A unique action of TCT on bone may help resolve the differences in potencies of CTs that led Deftos to write an editorial entitled, "There's something fishy or even fowl about the mammalian calcitonin receptor and its ligand"³⁵. A recent article by Bushinsky et al.³⁶ indicates that the ionic environment of bone exists in the organic phase, perhaps the bulk in the bone membrane. This organic phase is rich in sodium and potassium and both ions exceed the amount of bound calcium³⁶. They suggest "that the organic material in bone and not the mineral itself is responsible for the acute buffering of the

additional hydrogen ions during metabolic acidosis".

With regard to the kidney, TCT acts at various sites, modulating the excretion of Na, K, Cl, phosphate, as well as Ca in the kidneys of mammals. Exogenous CT stimulates secretion of these ions (see review by Horne et al.³⁷) and is a potent natriuretic agent in man³⁸, but these effects are usually observed at supraphysiological doses. On the other hand, lower concentrations of exogenous CT, closer to physiological doses, have been shown to have antidiuretic hormone (ADH) effects in the absence of ADH³⁹. These results suggest that the antidiuretic effect may be redundant or, as suggested by Elalouf et al.³⁹, may represent multiple hormonal effects. It has also been reported that exogenous CT, at high concentrations, stimulated the renin-angiotensin-aldosterone system that is so important in the control of water and electrolyte balance⁴⁰. It is possible that the effects of TCT may only be important under stressful situations related to water deprivation or loss such as mentioned above.

To summarize this hypothesis, it is important to recognize that all experiments performed on humans and laboratory animals occur under almost ideal conditions. Animal facilities are temperature and humidity controlled and usually animals are given free access to water. The excellent living conditions under which most experimental animals and humans exist do not provide situations whereby the homeostatic mechanisms of bone suggested by Frost³⁴ or of the kidney might become effective. Certainly, animals in the wild do not always have access to water and food and may be deprived of salts as well. Malnourished or isolated humans may suffer from significant dehydration. Such water and/or electrolyte deprivation could create conditions whereby TCT may protect the animal or human from death. Experiments involving water deprivation need to be carried out to test this hypothesis.

Hypothesis 3. TCT, a phosphate-conserving hormone

The premise of this hypothesis is that TCT originated in early vertebrates as a phosphate-conserving hormone and may retain that function to this day. It is suggested that in marine animals, by moving phosphate into bone and soft tissue, CT restricts the loss of this ion by gills and kidneys. The current hypothesis is an extension and modification of one put forward some twenty years ago^{12,41-44}; namely that, in mammals, TCT secreted during the feeding process functions to store calcium near bone surfaces for use during non-feeding periods. This storage of calcium is made possible by its combination with phosphate moved from ECF into bone fluid. We now propose that in mammals the original function of TCT to conserve phosphate has been adapted to conserve both calcium and phosphate. The combination of calcium and phosphate at bone surfaces temporarily stores calcium for use as needed during periods when ingested calcium is unavailable. We conclude that any physiological actions attributed to TCT must involve phosphate ions. Support for this relationship of TCT and phosphate is reviewed briefly below.

Hirsch et al.⁴⁵ reported that the injection of TCT-containing extracts from thyroid gland produced a hypophosphatemic effect in addition to its hypocalcemic action. Re-examination of their data indicated that the hypophosphatemic effect was greater than would be predicted if the hormone acted only by decreasing bone resorption. In a further study, the hypocalcemic effect of TCT was enhanced by the simultaneous administration of phosphate⁴⁶.

Several reports from Talmage's laboratory provide the following information: a) There is a direct correlation between the existing plasma phosphate level and the degree of hypocalcemia produced by TCT^{43,47}. b) Under specific conditions, the hypophosphatemic effect of TCT is independent of the hypocalcemic effect; i.e. the hypophosphatemic effect of TCT could be produced even in the absence of any effect on plasma calcium^{43,48}. c) The use of radionuclides of the two ions provided evidence that TCT moves phosphate into bone while it decreases the movement of calcium from bone⁴³. It was concluded from these studies, and those by Hirsch and Munson⁵, that the ability of the hormone to lower plasma phosphate is independent of the action of exogenous CT on osteoclastic bone resorption. In fact, it was further concluded that the hypocalcemia often seen after CT administration is not due to an inhibition of bone resorption but to the withholding of calcium by its union with phosphate at bone surfaces.

Supportive studies by Matthews et al.⁴⁹ demonstrated that TCT has rapid and specific effects on bone lining cells and osteocytes. These effects include the formation of a calcium-phosphate complex in the intercellular fluid spaces. In further studies it was shown that this complex is formed following the feeding of a calcium-containing meal to rats with functional TCT secreting glands, and that the complex disappears during non-feeding periods^{42,44}.

Norimatsu et al.⁵⁰ found that the administration of low doses of CT to rats increases deposition of phosphate on bone surfaces. In contrast, PTH decreases the deposition. Meyer and Meyer⁵¹ reported that CT administration to rats produces a hypophosphatemia accompanied by a significant uptake of phosphate by the liver.

Extensive studies reported over the same time period show a positive relationship between feeding periods and the secretion of TCT^{12,52-56}. Several intestinal secretagogues, specific for different mammals, stimulated TCT secretion. Gray and Munson⁴¹ suggested that TCT produces a temporary postprandial storage of calcium. Their conclusion was based on a study in which sham-operated or thyroidectomized rats were given calcium chloride by gavage or in a diet fed shortly after surgery. Changes with time in calcium content of plasma and urine were recorded. The relationship of feeding and gastrointestinal hormones has more recently been reviewed by Cooper⁵⁷.

Results from these studies prompted Talmage and his colleagues⁴²⁻⁴⁴ to propose that the action of TCT is to move phosphate postprandially into the bone environment where, combined with calcium, it provides a temporary storage of the latter ion for use in non-feeding periods. They suggested

that the advantage of this process to mammals is twofold. First, it provides a calcium source during fasting periods and thus reduces the amount of PTH needed to maintain plasma calcium and the need for mobilization of skeletal calcium. Secondly, it provides a continuous supply of calcium for the ongoing bone formation processes.

However, as mentioned earlier in this report, the absence of TCT secreting tissue does not appear to affect the growth, well being, or life expectancy of mammals (including humans). Therefore, the question is raised as to the physiological significance of postprandial storage of calcium by TCT. Is this a vestigial remnant of the evolutionary history of vertebrates or is it possible that the wrong experimental animals and/or circumstances have been studied? Regardless of the answer to the above questions, it can be postulated that any effect of TCT in mammals is the result of an adaptation of the earlier action of the hormone to conserve phosphate in those vertebrates living in a phosphate limited environment. Or an alternate explanation might be that the calcium storage sequence has nothing to do with calcium metabolism but is merely an indication of the role calcium plays in the conservation of phosphate.

This third hypothesis has been based on the premise that phosphate is directly involved in any action of CT, past or present. It has been most unfortunate that over the years, in the study of TCT, phosphate has been relegated to a secondary role in calcium homeostasis and bone remodeling. We propose that no real progress in understanding the role of this hormone will be made until its relationship to phosphate is thoroughly understood. This holds true whether the hormone is directly concerned with phosphate or merely uses this ion to achieve its goal.

Summary

In this editorial we present three hypotheses regarding roles for thyroidal CT. First, we discuss the possibility that TCT is a vestigial hormone. This concept is predominantly based on the fact that no deficiency or excess syndrome for TCT has been identified. The second hypothesis suggests that the action of TCT, such as the action of CT in marine fish, might involve functions that are not related to calcium metabolism. The suggestion is made that in mammals TCT may restrict water loss, perhaps by regulating ion distribution or acid-base balance during emergency situations in which life is threatened. The third hypothesis suggests that TCT secreted at time of eating induces the formation of a calcium-phosphate colloid complex through the movement of phosphate. Such stored calcium would be available for use during non-feeding periods. Alternately, the hormone may merely use calcium to conserve phosphate when the latter ion is limited in the diet. Experiments to obtain evidence for the last two hypotheses need to be performed under controlled conditions with limited amounts of water, dietary phosphate and/or calcium. These experiments might support hypothesis 2 or 3, or both, but the effects may be too small to

be physiologically significant. Such findings would suggest that TCT is, or is becoming, a vestigial hormone.

The primary purpose of this editorial is to offer several hypotheses to be considered for the functional status of TCT. An even more important purpose is to stimulate investigators not only to assess these proposed hypotheses but also to propose other possible physiological functions for TCT, an important therapeutic agent with an uncertain physiological role.

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