Thyrocalcitonin and the Response to Parathyroid Hormone *

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Summary. 1) In the absence of the thyroid gland, the infusion of parathyroid hormone leads to a prompt rise in plasma calcium and to prompt increase in the rate of excretion of calcium in the urine.

2) In the presence of the thyroid gland, the parathyroid hormone-induced rise in plasma calcium is less marked; the rate of urinary calcium excretion falls initially and rises only after 20 to 30 hours of continuous parathyroid hormone infusion.

3) The infusion of exogenous thyrocalcitonin along with the parathyroid hormone into a thyroparathyroidectomized animal leads to a pattern of response similar to that seen in the animal with an intact thyroid gland.

4) Thyrocalcitonin has little apparent effect upon the immediate changes in renal function induced by parathyroid hormone.

5) We conclude that bone is a major site of action of thyrocalcitonin and that it probably inhibits bone resorption.

Introduction

Since the work of Sanderson, Marshall, and Wilson (1) and that of Copp and co-workers (2), evidence has rapidly accumulated indicating that the thyroid gland is the source of a hypocalcemic principle (3-8). This principle, termed "thyrocalcitonin" by Hirsch, Gauthier, and Munson (3), has recently been isolated from porcine thyroid tissue and has been shown to be a polypeptide (9). Its isolation has allowed us to examine the relationship between its activity and that of the parathyroid hormone, and to determine its role in modulating the response of the parathyroidectomized rat to the constant infusion of parathyroid hormone.

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Methods

Male Holtzman rats, weighing approximately 100 g, were given 100 U of vitamin D₂ by oral intubation and maintained on a stock diet containing adequate quantities of this vitamin. When they reached 120 to 140 g, they were either parathyroidectomized or thyroparathyroidectomized by surgery. The influence of the long term iv perfusion of purified parathyroid hormone in all animals, or of both parathyroid hormone and purified thyrocalcitonin in the thyroparathyroidectomized group, upon the plasma calcium, the urinary excretion of phosphate, calcium, and magnesium, and the clinical course of the animal was observed. The technique of long term perfusion of the rat was developed as a modification of that described by Cotlove (10).

Blood samples were obtained by making a clean slash over the dorsum of the foot with a new sharp razor blade and aspirating approximately 0.3 ml with a 1-ml heparinized tuberculin syringe through a no. 20 needle.

All animals were perfused with a solution containing dextrose 4%, and calcium 5 mM, magnesium 5 mM, sodium 20 mM, and potassium 2.5 mM, all as the chloride salts. The perfusion was maintained for a period of 16 hours after surgery before measurements of urinary volume and calcium content were begun. Collections were made during a control period of at least 10 hours before parathyroid hormone (5 µg per hour) or thyrocalcitonin

FIG. 1. THE RATE OF EXCRETION OF PHOSPHATE, CALCIUM, AND MAGNESIUM IN THE URINE OF A) PARATHYROIDECTOMIZED AND B) THYROPARATHYROIDECTOMIZED RATS BEFORE AND DURING THE CONSTANT INFUSION OF PARATHYROID HORMONE (PTH).

(25 µg per hour) or both were added to the perfusate. Hormone infusion was continued either until the animal died with hypercalcemia and oliguria or for 60 to 65 hours. The thyroidectomized animals received 0.01 mg of *l*-thyroxine every 12 hours. Urine was collected continuously in a fraction collector in 2-hour periods. Blood samples were obtained in all animals during the control period, 15 hours, and in most instances, approximately 25 hours after the start of hormone infusion. In animals that survived, additional samples were taken 45 hours and 60 hours after the start of hormone infusion.

In other groups of animals the effects of thyrocalcitonin administration upon the plasma calcium and phosphate concentrations were determined. The effect of the administration of actinomycin D upon this response was studied. All animals were maintained on a low calcium diet for 5 days. One group was given actinomycin D, 15 µg per day for 3 days. Both groups were then injected with 5 mg of crude thyrocalcitonin, and a plasma sample was obtained by heart puncture under light ether anesthesia 1½ hours later.

The concentrations of urinary calcium and phosphate were measured as previously described (11). After deproteinization, we measured plasma calcium by flame absorption spectroscopy in the presence of 10,000 parts per million of lanthanum in order to prevent phosphate interference (12). Magnesium was measured by atomic absorption spectroscopy (13). The purified thyrocalcitonin used in this study was that of Tenenhouse, Arnaud, and Rasmussen (9), and the purified parathyroid hor-

mone was the preparation of Hawker, Glass, and Rasmussen (14).

Results

The typical pattern of response of a parathyroidectomized rat to the long term infusion of parathyroid hormone is shown in Figure 1A. The initial response was characterized by a prompt phosphaturia, accompanied by a decreased rate of calcium and magnesium excretion. Only after 17 to 22 hours of continuous infusion did calcium excretion rise and significant hypercalciuria develop. In contrast, the infusion of hormone into a thyroparathyroidectomized animal (Figure 1B) led to a rise in calcium excretion within 2 hours as well as the usual fall in magnesium excretion and immediate phosphaturia.

To further define this striking difference in the pattern of urinary calcium excretion, we measured the plasma calcium concentrations and rates of urinary calcium excretion in three different types of animal: thyroparathyroidectomized rats given 5 µg per hour of parathyroid hormone, parathyroidectomized animals given parathyroid hormone, and thyroparathyroidectomized rats given both parathyroid hormone and thyrocalcitonin.

FIG. 2. THE PLASMA CALCIUM CONCENTRATION (○-----○) AND RATE OF URINARY CALCIUM EXCRETION (●-----●) BEFORE AND DURING THE INFUSION OF PURIFIED PARATHYROID HORMONE IN A) A THYROPARATHYROIDECTOMIZED RAT (PTX-TX), B) A PARATHYROIDECTOMIZED RAT WITH AN INTACT THYROID, AND C) A THYROPARATHYROIDECTOMIZED RAT RECEIVING A SIMULTANEOUS INFUSION OF THYROCALCITONIN (TCT). Oliguria developed after 16 hours of parathyroid hormone infusion in rat A and after 48 hours in rat B. No oliguria was seen in rat C.

Typical responses in each type of animal are shown in Figure 2.

The thyroparathyroidectomized animal receiving only parathyroid hormone (Figure 2A) had a prompt rise in plasma calcium and developed a prompt increase in urinary calcium excretion. The latter was sustained for 12 to 14 hours and then fell rapidly due to the development of profound oliguria, which was followed in another 3 to 4 hours by death. At the time of death profound hypercalcemia was evident (18.5 mg per 100 ml). The cause of oliguria was apparent upon autopsy; both kidneys exhibited gross nephrocalcinosis.

The thyroparathyroidectomized animal receiving the simultaneous infusion of both hormones (Figure 2C) showed a moderate rise in plasma calcium (9.4 to 11.1 mg per 100 ml) accompanied by a significant ($p < 0.02$) decrease in urinary calcium excretion, which remained below the control rate for the first 28 to 30 hours of hormone infusion and then rose rather promptly to rates considerably in excess of the control rates in conjunction with a further rise in plasma calcium. The infusion was discontinued after 76 hours. No oliguria had developed, and the kidneys appeared grossly normal.

The infusion of parathyroid hormone into the animal with an intact thyroid gland (Figure 2B) led to a pattern of response qualitatively similar to that observed in the thyroparathyroidectomized animal given both hormones (Figure 2C). An initial slight rise in plasma calcium accompanied by a fall in urinary calcium excretion was followed in 35 to 40 hours with a further rise in plasma and urinary calcium. In this particular animal oliguria eventually developed after 55 hours.

Complete perfusion studies measuring plasma calcium and the rates of excretion of calcium, phosphate, and magnesium were carried out in four different groups of animals: I, thyroparathyroidectomized animals given 5 μ g of parathyroid hormone per hour; II, thyroparathyroidectomized animals given 1 μ g of parathyroid hormone per hour; III, parathyroidectomized animals given 5 μ g per hour; and IV, thyroparathyroidectomized animals given 5 μ g of parathyroid hormone and 25 μ g of thyrocalcitonin per hour. The results are summarized in Table I. The plasma calcium values before and at 15, 25, 45, and 60 hours of hormone infusion are recorded, as well as the rates

of urinary excretion (micromoles per minute) of calcium, phosphate, and magnesium before and at intervals during hormone infusion. The values recorded are the means for groups of five rats except in group IV, in which only four animals were employed. The urinary values in group I are not recorded for hour 25 and are highly variable at hour 20 because these animals all developed oliguria some time between 16 and 22 hours (average, 18.7 hours). None of the animals in group II and only one in group IV developed oliguria during 60 hours of parathyroid hormone infusion, but three of the five animals in group III developed it between 40 and 50 hours of perfusion. Although there was some spread in the values within each group, the control values of the different groups agreed rather well, and there were significant differences between the responses of group I and group II; between those of group I, and groups III and IV; and between those of group III and group IV.

The results indicate that either endogenous (animals with thyroid glands intact) or exogenous (with thyroid glands absent) thyrocalcitonin alters the response of parathyroidectomized animals to the infusion of parathyroid hormone. The most striking alterations were observed in three parameters: urinary calcium, urinary phosphate, and plasma calcium.

Thyrocalcitonin suppressed the rise in plasma calcium and the enhanced excretion of calcium seen after parathyroid hormone infusion into thyroparathyroidectomized animals (compare the 0-, 5-, and 15-hour values between group I and groups III and IV). In both the latter groups, the infusion of parathyroid hormone was followed by an initial fall in the rate of urinary calcium excretion compared to a significant and progressive rise in group I. In the animals receiving the infusion of thyrocalcitonin (group IV), the urinary calcium excretion remained very low during the first 25 hours of perfusion, whereas in those animals with intact thyroid glands, the rates of calcium excretion were never quite so low as those seen in group IV animals, and they returned to control values within 15 to 20 hours after initiation of perfusion. This difference was related to the fact that plasma calcium concentrations rose sooner and reached higher values in the animals in group III as compared to those in group IV, i.e., the

TABLE I

The changes in plasma calcium and in rates of excretion of urinary electrolytes during parathyroid hormone infusion into thyroparathyroidectomized and parathyroidectomized rats

	Hours of parathyroid hormone infusion							
	0	5	10	15	20	25	45	50
Group I (TPX-5)*								
Plasma Ca, mg/100 ml	8.2 ± 0.3†			15.3 ± 1.2		17.5 ± 2.8‡		
Urinary Ca, μmole/min	0.06 ± 0.02	0.12 ± 0.02	0.15 ± 0.03	0.22 ± 0.03	0.09 ± 0.05			
Urinary HPO ₄ , μmole/min	0.24 ± 0.08	1.08 ± 0.21	0.83 ± 0.18	0.72 ± 0.20	0.48 ± 0.22			
Urinary Mg, μmole/min	0.15 ± 0.04	0.07 ± 0.03	0.02 ± 0.02	0.07 ± 0.03	0.11 ± 0.05			
Group II (TPX-1)*								
Plasma Ca, mg/100 ml	7.9 ± 0.3		8.7 ± 0.4			9.1 ± 0.4	10.3 ± 0.6	11.2 ± 0.6
Urinary Ca, μmole/min	0.05 ± 0.02	0.01 ± 0.01	0.05 ± 0.04	0.13 ± 0.03	0.20 ± 0.06	0.18 ± 0.07	0.24 ± 0.07	0.28 ± 0.9
Urinary HPO ₄ , μmole/min	0.21 ± 0.06	0.54 ± 0.11	0.43 ± 0.07	0.28 ± 0.06	0.22 ± 0.06	0.33 ± 0.08	0.47 ± 0.12	0.11 ± 0.11
Group III (PX-5)*								
Plasma Ca, mg/100 ml	7.6 ± 0.4			12.4 ± 0.3		11.5 ± 0.6	18.0 ± 1.1	16.5 ± 2.3‡
Urinary Ca, μmole/min	0.07 ± 0.03	0.03 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.06 ± 0.03	0.09 ± 0.04	0.15 ± 0.04	
Urinary HPO ₄ , μmole/min	0.18 ± 0.06	0.96 ± 0.11	0.61 ± 0.09	0.43 ± 0.07	0.32 ± 0.07	0.22 ± 0.07	0.34 ± 0.13	
Urinary Mg, μmole/min	0.17 ± 0.03	0.09 ± 0.03	0.09 ± 0.04	0.13 ± 0.03	0.14 ± 0.04	0.07 ± 0.05	0.23 ± 0.09	
Group IV (TPX-5 + TCT)*								
Plasma Ca, mg/100 ml	8.2 ± 0.4			9.3 ± 0.5	10.4 ± 0.6		14.4 ± 0.8	15.0 ± 0.8
Urinary Ca, μmole/min	0.05 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.03	0.14 ± 0.03	0.17 ± 0.05
Urinary HPO ₄ , μmole/min	0.18 ± 0.05	0.71 ± 0.13	0.48 ± 0.06	0.35 ± 0.12	0.32 ± 0.11	0.23 ± 0.13	0.21 ± 0.08	0.33 ± 0.08
Urinary Mg, μmole/min	0.20 ± 0.04	0.07 ± 0.04	0.07 ± 0.04	0.16 ± 0.08	0.20 ± 0.08	0.33 ± 0.11	0.25 ± 0.09	0.31 ± 0.09

* TPX-5 = thyroparathyroidectomized animals receiving 5 μ g parathyroid hormone per hour; TPX-1 = thyroparathyroidectomized animals receiving 1 μ g parathyroid hormone per hour; PX-5 = parathyroidectomized animals receiving 5 μ g parathyroid hormone per hour; TPX-5 + TCT = thyroparathyroidectomized animals receiving 5 μ g of parathyroid hormone and 25 μ g of thyrocalcitonin per hour.

† Each value is the mean $\pm$ standard error determined upon five animals, except in group IV, in which only four animals were studied.

‡ All or several of the rats in these groups had developed significant oliguria. Hence urinary values are not recorded although plasma values are.

infusion of thyrocalcitonin at a rate of 25 μ g per hour suppressed the hypercalcemic and hypercalciuric effects of parathyroid hormone infusion to a greater degree than did the presence of the animal's own thyroid gland.

The changes in urinary phosphate excretion in the three groups, I, III, and IV, were also dif-

ferent. All showed an initial sharp increase in phosphate excretion within the first 2 hours of parathyroid hormone infusion. Peak rates of excretion at this time were the following: group I, 1.2 ± 0.25 μ moles per minute; group III, 1.1 ± 0.21 μ moles per minute; and group IV, 0.8 ± 0.2 μ moles per minute. The animals in group I had a high rate of excretion during the next 15 hours. The lower values seen at 20 hours in this group are probably a reflection of the oliguria these animals developed. In the other two groups (III and IV), the initial high rates of phosphaturia were followed by a gradual fall, so that the rates had returned to control values by 20 to 30 hours, usually followed by a rise in the later hours. Of particular note is the fact that the maximal rates of phosphate excretion as well as the rates at 5 and 10 hours were significantly less in the animals infused with thyrocalcitonin (group IV) than those in the animals with intact thyroid glands (group III).

Studies were carried out in thyroparathyroidectomized animals employing different rates of parathyroid hormone perfusion in order to define the sensitivity of this type of animal to this hormone. Typical responses of individual thyro-

FIG. 3. THE PLASMA CALCIUM CONCENTRATION (○-----○) AND RATES OF EXCRETION OF CALCIUM (○-----○) AND PHOSPHATE (●-----●) IN THE URINE OF TWO DIFFERENT THYROPARATHYROIDECTOMIZED RATS BEFORE AND DURING THE PERFUSION OF A) 3 μ g PER HOUR AND B) 1 μ g PER HOUR OF PARATHYROID HORMONE.

parathyroidectomized rats to the infusion of 3 μg per hour and 1 μg per hour of parathyroid hormone are shown in Figure 3. The results with 3 μg per hour were quite comparable to those seen in rats receiving 5 μg per hour except that the initial fall in the urinary excretion of calcium was clearly evident with the smaller dose. This was followed by a significant rise with oliguria and death. Four animals were given hormone at a rate of 3 μg per hour. All showed a similar pattern of response. The only significant difference was an initial fall in calcium excretion during the first 3 to 5 hours of parathyroid hormone infusion. The mean rate of calcium excretion at 5 hours was 0.05 ± 0.02 μmole per minute compared to a control value of 0.08 ± 0.02 μmole per minute. This value of 0.05 ± 0.02 was significantly less ($p < 0.02$) than that seen in animals receiving 5 μg per hour of parathyroid hormone (0.112 ± 0.02 μmole per minute, Table I). The remainder of the values for this group of animals were not significantly different from those seen in the animals given parathyroid hormone at a rate of 5 μg per hour. The infusion of 1 μg per hour was tolerated for 55 hours without deleterious effects upon the animal. A significant phosphaturia was produced by this level of hormone, although there was considerable fluctuation in spite of a nearly constant rate of urine flow (not charted). Similarly, this dose of hormone produced an initial significant decrease ($p < 0.01$) in rate of calcium excretion followed in a matter of 5 to 15 hours by a sustained but fluctuating increase in rate of excretion. A summary of the results obtained with a total of five animals perfused with parathyroid hormone at a rate of 1 μg per hour is shown in Table I. The results are similar to those depicted in Figure 3.

The fact that thyrocalcitonin prevented parathyroid hormone-induced calcium mobilization raises the interesting possibility that thyrocalcitonin acted by inhibiting bone resorption. Since previous study had shown that actinomycin D inhibits, to a considerable degree, the effect of parathyroid hormone upon bone mobilization (15, 16), we studied the effect of actinomycin D upon thyrocalcitonin response.

Thyrocalcitonin exerted its usual effect in actinomycin D-treated animals. During the course of the present study a similar finding was reported

by Tashjian (17). The results of the two studies agree that thyrocalcitonin exerts its hypocalcemic action in the presence of a sufficient concentration of actinomycin D to prevent the mobilization of significant calcium from bone by parathyroid hormone, and that in fact the animals are somewhat more sensitive to thyrocalcitonin after treatment with this antibiotic.

Discussion

Our results indicate that the thyroid gland plays an important role in protecting rats from the hypercalcemic action of parathyroid hormone. This function of the thyroid can be replaced by the infusion of exogenous thyrocalcitonin, leading to the logical conclusion that thyrocalcitonin is elaborated by the thyroid gland in response to hypercalcemia.

It becomes of considerable interest to determine whether the nephrocalcinosis observed in the thyroidectomized animals given parathyroid hormone is due solely to the hypercalcemia, or whether the parathyroid hormone has a more specific effect in the pathogenesis of this nephrocalcinosis. The only information bearing on this point is the report of Caulfield and Schrag (18) in which they concluded that parathyroid hormone does have a specific pathogenic role unrelated to its hypercalcemic action. This point should be further investigated. Conversely, it should be of some practical interest to determine whether thyrocalcitonin has a specific protective effect at the level of the renal tubule or merely protects the animal by the control of hypercalcemia.

It has generally been thought that the effect of parathyroid hormone upon bone resorption is slow in onset, although Talmage and his colleagues (19) have shown changes in calcium mobilization within 2 hours after parathyroidectomy. Our results confirm and extend these observations, indicating that, in the absence of the thyroid gland or of exogenous thyrocalcitonin, parathyroid hormone infusion leads to the prompt mobilization of calcium from bone. The effect is sufficiently rapid (within 30 to 60 minutes) that it obliterates the usual decrease in calcium excretion seen upon initiation of parathyroid hormone infusion (Figure 1A and Table I).

The marked delay in parathyroid hormone-induced calcium mobilization and the less sus-

tained phosphaturia seen in animals with intact thyroid glands (Figures 1A and 2B, Table I) and in those receiving exogenous thyrocalcitonin (Figure 2C, Table I) suggest that bone is a most important site of thyrocalcitonin action. Other investigators have come to a similar conclusion (7, 19-21). They have proposed that thyrocalcitonin acts by suppressing bone resorption. Our results are consistent with this conclusion. However, none of the data are sufficient to rule out the possibility that thyrocalcitonin also promotes calcium or mineral accretion. In any case, there are several interesting aspects to thyrocalcitonin action. This hormone continues to exert its effects in parathyroidectomized animals (17) and is effective in animals treated with actinomycin D (17). The administration of this antibiotic to parathyroidectomized animals suppresses the mobilization of calcium from bone usually observed after parathyroid hormone infusion (15-17). However, it is clear from both *in vivo* (22) and *in vitro* (23) observations that actinomycin does not suppress the immediate effects of parathyroid hormone upon calcium mobilization. Thus the present data (Table I) do not help to define the relationship between the actions of thyrocalcitonin and parathyroid hormone.

The most compelling evidence for an effect of thyrocalcitonin upon bone resorption is its ability to block parathyroid hormone action (Figure 2C, Table I) and the data of Kohler and Pechet (21) indicating that thyrocalcitonin infusion leads to a striking decrease in hydroxyproline excretion. Their data using proline-¹⁴C show that this hormone has little effect upon collagen synthesis but inhibits collagen degradation. An effect upon resorption is also evident from the studies of the action of this hormone upon bone grown in tissue culture (19, 20).

A further study of the effects of thyrocalcitonin upon various parameters of bone metabolism is clearly in order; the results of such studies may lead to new concepts concerning physiologic models of bone metabolism. Most of the studies of the effect of parathyroid hormone upon various parameters of bone metabolism [reviewed by Nichols (24)] have been carried out in animals with intact thyroid glands. Since all of the studies with thyrocalcitonin lead to the conclusion that it acts upon bone, the interpretation of these

changes in bone metabolism, seen after parathyroid hormone administration, is open to question. The experiments should be repeated in thyroparathyroidectomized animals to clarify the role thyrocalcitonin may play in the observed metabolic alterations.

The fact that in bone these two hormones appear to have antagonistic effects should not overshadow the fact that there is no such antagonism in other end organs. In our study, there was little difference in the initial phosphaturia induced by parathyroid hormone in the presence (Figure 1; Table I, group III) and absence (Figure 1; Table I, group I) of the thyroid gland, and even the simultaneous infusion of a large dose of thyrocalcitonin (group IV) did not suppress this initial renal effect of parathyroid hormone. Furthermore, parathyroid hormone given in smaller doses to animals without parathyroid or thyroid glands (Figure 3) led to an initial retention of calcium by the kidney. This was also seen in the parathyroidectomized animals (thyroids intact, group III) given parathyroid hormone. In both instances plasma calcium was rising. Thus thyrocalcitonin does not alter the renal response to parathyroid hormone. The later differences in phosphate excretion between group I and groups III and IV (Table I) are probably related to the suppression of phosphate mobilization from bone with a consequent decline in phosphate excretion (groups III and IV). This difference in end organ responsiveness accounts for the fact that although these two hormones have opposing effects upon plasma calcium, they both lower plasma phosphate (7, 9), parathyroid hormone primarily by an effect upon the kidney, thyrocalcitonin apparently by an effect upon bone. The integration of these responses means that by the proper adjustment of the secretion rates of these two hormones, plasma phosphate can be varied relatively independently of plasma calcium.

These conclusions raise a point of considerable clinical interest. Two distinct forms of hyperparathyroidism have been recognized, one accompanied by bone disease and another without apparent bone disease (25). Several different proposals have been put forward to account for this apparent division. These have ranged from that of Dent (26), who considered the parathyroid gland to secrete two distinct hormones, to that

of Rasmussen, who assumed that the dietary intake of inorganic phosphate or some other key nutrient may account for the difference (27). The former explanation seems unlikely in view of the evidence that a single molecule possesses all the known activities attributable to parathyroid hormone (28). The second explanation has little direct support. An alternative to both emerges from the present observations. As can be seen from our data, thyrocalcitonin does not alter the renal effects of parathyroid hormone, whereas it does suppress the effects of this hormone upon bone resorption. Thus the presence or absence of bone disease in hyperparathyroidism may depend upon the ability of the thyroid gland to respond to hypercalcemia by increasing its production of thyrocalcitonin. In the patients without bone disease, compensatory hyperactivity of the thyroid is sufficient to suppress the bone-resorbing activity of the increased circulating parathyroid hormone. Nevertheless, because of the ability of parathyroid hormone to induce calcium retention and phosphate excretion by the kidney, and promote calcium absorption from the gastrointestinal tract, hypercalcemia would be present in these patients with hyperparathyroidism. This proposal, if correct, raises the ever intriguing question of why the secretion of thyrocalcitonin does not increase sufficiently to compensate for parathyroid overactivity in all patients with hyperparathyroidism.

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