Thyrocalcitonin: Effects on Calcium Kinetics in the Rat. (31443)

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We have suggested that hypocalcemia following thyrocalcitonin (TCT) administration is due to an inhibition of bone catabolism in the fasted rat(1). Hypocalcemia can be considered as the final expression of a number of changes affecting the parameters of calcium metabolism. This paper concerns quantitation of the pathways of calcium metabolism in rats treated with a long-acting TCT preparation and maintained on a normal diet, in order to establish the effects of the hormone on the target organs and to distinguish primary from secondary effects.

Materials and methods. Wistar male rats, 60 days of age and weighing 150 g, were divided into 2 groups, 15 controls and 15 TCT-treated. Controls received subcutaneously 0.8 ml of acetate buffer, 0.1 *M*, pH 5.4, and the other group received 320 U(2) of purified long-acting TCT preparation(2,3), every 8 hours for 3 days. Preliminary experiments have shown that under these conditions low plasma calcium levels could be maintained constant for three days.

Calcium metabolism was studied with a technique combining balance data and radiocalcium measurements. We have previously shown that calcium metabolism can be described in terms of various parameters, which can be calculated from a detailed analysis of the integrated plasma specific activity curve and from the amounts of calcium ingested and excreted by way of urine and feces(4). Furthermore, different experimental conditions were reflected by changes in the values of these parameters—for example rickets(4) and increase in age(5). A single tracer dose of Ca^{45} (50 μC) was injected intravenously 2 hours after the first administration of either TCT or buffer. To study the influence of food intake various amounts of a balanced diet containing 6 mg/g each of calcium and phosphorus were given; calcium intake varied between 20 and 80 mg/day (mean intake for the 2 groups, 60 mg/day).

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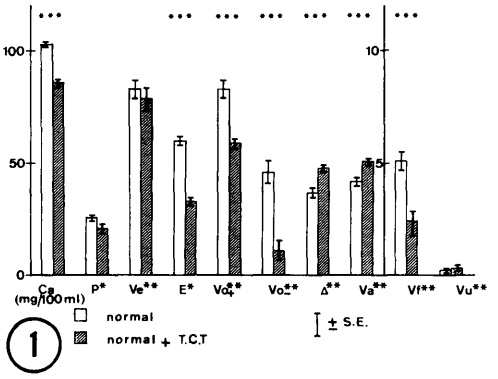
Statistical treatment of data: the means of all the parameters were compared by Student test except for the balance (Δ) and amount of calcium absorbed by the gut (Va), which were compared by covariance analysis. The absorption coefficient a being the ratio Va/Vi (Vi = ingested calcium) was computed by the coefficient of regression between calcium absorbed and calcium ingested.

Results. Fig. 1 shows the means and standard errors of the mean for the main parameters measured in the 2 groups. Thyrocalcitonin lowers Ca, (plasma calcium), Vo^- (bone catabolism), Vo^+ (bone anabolism), Vf (endogenous fecal calcium), E (slowly exchangeable calcium compartment, 8 hr). There is a highly significant rise in Va, calcium absorbed during digestion, and Δ (calcium balance). All other parameters remained unchanged: P (exchangeable calcium, 2 hr), Ve (rate of exchange between the calcium compartments P and E), Vu (urinary calcium).

Fig. 2 illustrates the regression curves Va/Vi for the 2 groups. The difference between the regression coefficients is highly significant.

Discussion. Our present results are in agreement with our previous interpretation of changes in plasma calcium specific activity in the fasted rat, following injection of the TCT hormone(1). In the fasted animal, where Va is practically suppressed, the dilution of the radioactivity of the pool by unlabelled calcium can only be due to bone catabolism Vo^-. We found that following injection of the hormone there was the expected fall in calcemia but the specific radioactivity of the pool remained constant. It was considered that this could only be due to an inhibition of bone catabolism Vo^-.

In the experimental conditions used in the present study, thyrocalcitonin maintains a low plasma calcium level for 3 days. This has been interpreted as being due to the dramatic inhibition of Vo^- (bone catabolism), which



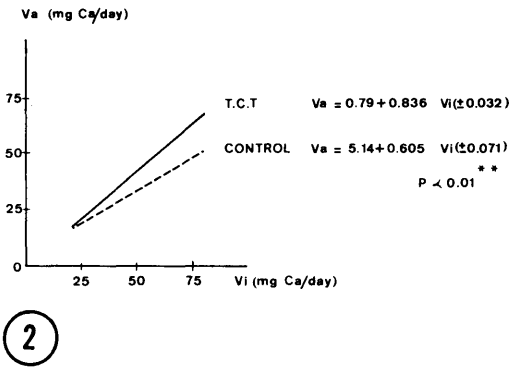


FIG. 1. Effect of long-acting thyrocalcitonin administration on main parameters of calcium metabolism. *** $P < 0.001$; ** expressed as mg/day; * expressed as mg.

FIG. 2. Effect of long-acting thyrocalcitonin on absorption of calcium by the gut.

is decreased 75%. This must be the primary effect as all other parameters involved would tend to raise plasma calcium level: reduction of Vo^+ (bone anabolism) to 2/3, increase of Va (calcium absorption during digestion) and decrease of Vf (fecal endogenous calcium). The excretion of calcium through the kidney is unaffected.

With regard to the two compartments of the exchangeable calcium pool, only the slower exchangeable compartment E is statistically decreased. No change could be demonstrated on the faster exchangeable compartment P, which includes plasma calcium.

As to the faster exchangeable calcium compartment, P, the decrease of the mean value is 4.47 mg; this decrease, though not statistically significant, is of the same order as that of the diminution of the plasma calcium, which is reflected by the hypocalcemia since the plasma volume is unchanged by TCT(1).

The balance or net gain of the animal (Δ) can be equated to net gain in bone in the case of calcium, or more precisely to: $\Delta = Vo^+ - Vo^-$(6). Sustained TCT hypocalcemia is accompanied by an increase in the net gain of calcium.

This increase in Δ could be achieved by several combinations of changes in the processes, Vo^+ and Vo^-, provided that the resulting difference between the two is increased. In fact, there is a moderate reduction in bone anabolism, Vo^+, which would tend to prevent excessive calcium deposition in bone. This is accompanied, however, by a very much greater decrease in bone catabolism, Vo^-, and it is suggested, therefore, that the increase in net gain under the action of TCT is due to this marked inhibition of bone catabolism.

Thyrocalcitonin increases the amount of calcium absorbed by the gut. This is reflected by a higher coefficient of absorption $\alpha = Va/Vi$, which is 84% as compared with 60% for the controls, for calcium intakes varying from 20 to 80 mg/day. This observation made on intact rats could be explained by an enhanced parathyroid activity in response to hypocalcemia, since in an earlier study we found that parathyroid extract was able to increase the absorption coefficient in thyroparathyroidectomized rats(7). We are not able to rule out this possibility, since the results of our experiments with repeated TCT injections in thyroparathyroidectomized rats were not statistically significant(3). In any event the two processes responsible for the entry of calcium into the pool, Vo^- and Va, are linked together and the decrease of Vo^- is accompanied by an increase in Va.

Endogenous fecal calcium output (Vf) is diminished by half; this could be explained on the basis of the enhanced intestinal absorption and does not necessarily mean a decreased secretion of calcium in the digestive juice, Vd(8). Vd can be calculated from α and Vf using the relation:

$$Vd = \frac{Vf}{1 - a}$$

and is equal to 12.5 mg/day for the controls and 14.7 mg/day for the TCT-treated animals.

The rise in urinary calcium output under TCT treatment is not significant; this means that the kidney is not involved in the mechanism of TCT-induced hypocalcemia. This finding is consistent with the observation that nephrectomy does not prevent the hypocalcemic response to TCT(9,10).

Conclusion. Thyrocalcitonin is able to produce and maintain a low plasma calcium level in an intact animal fed a normal diet. The marked inhibition of bone catabolism by thyrocalcitonin would suggest that this is the main mechanism involved in the lowering of the plasma calcium. Since bone anabolism is also decreased (a factor which would tend to raise plasma calcium) any contribution to the lowering of plasma calcium from this process would appear unlikely. Thyrocalcitonin administration results in an increased calcium balance. The utilization of dietary calcium by the gut is enhanced by TCT. In summary, thyrocalcitonin is a *calcium-sparing hormone.*

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Inhibition of J-128 (Osgood) Cell Culture by Azaserine.* (31444)

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Azaserine interferes with several glutamine catalyzed enzyme reactions, and it has been concluded that the primary site of action involves inhibition of conversion of formylglycineamide-ribotide to formylglycineamidine-ribotide(1). This assumption has been made on the basis that amino-imidazole carboxamide (AICA) will reverse the inhibitory effect of azaserine on the growth of *E. coli* cells (2). This interpretation must be questioned, however, as several amino acids also reverse the inhibitory effect of the drug(2). In a preliminary report, we presented evidence that inhibition of the growth of an established cell line in tissue culture by azaserine was not reversed by addition of hypoxanthine, adenine or AICA to the medium(3). This cell line (Osgood J-128) utilized hypoxanthine, adenine or AICA in preference to formation of purine by *de novo* synthesis, thus it was apparent that the drug interfered with some biochemical reaction other than blockade of *de novo* purine synthesis. The data suggested that azaserine did not exert its action by blocking conversion of xanthylic to guanylic acid, a reaction which also has been demonstrated to be inhibited by azaserine *in vitro*(4). However, the evidence was not clear cut since the culture converted guanine to guanylic acid at

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