The Effect of Calcitonin on ^{32}P Disappearance from Plasma in Parathyroidectomized and Nephrectomized Rats[1] (36915)

ROY V. TALMAGE AND J. J. B. ANDERSON[2]

Division of Orthopaedic Surgery, University of North Carolina, School of Medicine, Chapel Hill, North Carolina 27514

Recently it has been shown that one of the early actions of calcitonin is to cause a rapid movement of phosphate from plasma (1). This finding is in contrast to the currently held postulate that the hypophosphatemia is caused by a reduction of the entry of phosphate into plasma from bone due to suppression by calcitonin of bone resorption.

The conclusion that calcitonin increased the rate of movement of phosphate out of plasma in intact rats was based on the finding that the hormone altered the rate of disappearance of ^{32}P from rat plasma to the same degree as that for inorganic P_i when the hormone and the radionuclide were administered within 5 min of each other. Thus the specific activity values of the disappearance curves of calcitonin-treated and control rats were identical.

The experiments reported above were performed using only intact rats, namely those with functional parathyroids and kidneys. The present experiments were carried out to determine whether this effect on phosphate and ^{32}P was caused in part by the action of parathyroid hormone on renal excretion of phosphate secondary to a fall in plasma calcium. The results from intact rats were therefore compared to those obtained by injecting calcitonin into rats at various times after parathyroidectomy and nephrectomy.

Materials and Methods. The experiments reported in this study utilized approximately 60 Zivic-Miller Labs male rats. The salmon calcitonin (SCT) was kindly provided by D. H. Copp.

A standard dose of 25 MRC mU in 0.25 ml was administered subcutaneously. All injections were made from the same lot of calcitonin (300 MRC U/mg) assayed at the Medical Research Council of England. Some control animals were injected with carrier, but this was discontinued since no effect of this agent could be observed.

For these experiments calcitonin was injected 5 min prior to the intraperitoneal injection of 10 to 15 μCi each of ^{32}P and ^{45}Ca. To evaluate the disappearance rates of radioisotopes from plasma, data were calculated as percentage of injected dose per milliliter of plasma. Determination of the radioactivity (^{45}Ca and ^{32}P) in each sample was calculated using a multichannel Beckman liquid scintillation spectrometer.

Blood samples were obtained 1 hr after SCT injection. Determinations of calcium and inorganic phosphate were made on 20 μl aliquots of plasma by the methods of Hill (2) and Chen, Toribara and Warner (3), respectively; 50 μl aliquots were used for determination of radioactivity. Both inorganic phosphate and inorganic ^{32}P were determined on trichloroacetic acid (TCA) filtrates.

All surgical procedures were done under light ether anesthesia. In one group, parathyroid glands were transplanted to the eye 2 or 3 wk before utilization. In this group thyroidectomy was performed 18 hr prior to use. For those animals acutely thyroparathyroidectomized or nephrectomized, the animals were given 1 hr to recover from anesthesia before use.

Results. The groups studied included control rats; rats thyroidectomized but containing functional parathyroid transplants; rats acutely thyroparathyroidectomized; rats parathyroidectomized 18 hr prior to use; and rats acutely nephrectomized. Since the previous report (1) had indicated that the maximum difference was obtained in 1 hr after isotope administration, only this time period

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[2] On leave from University of Illinois, College of Veterinary Medicine.

TABLE I. Plasma Calcium and Phosphate Values 1 hr After SCT Injection.[a]

	Controls	TX 18 hr	PTX 18 hr	Acute TPTX	Acute Nephx
Av animal wt (g)	185	219	153	168	172
Plasma values					
Calcium (mg/100 ml)[b]					
Non-injected	9.9 ± 0.2[c]	9.8 ± 0.04	6.0 ± 0.31	8.5 ± 0.23	10.5 ± 0.18
SCT-injected	7.8 ± 0.2	7.7 ± 0.02	4.5 ± 0.13	6.2 ± 0.12	8.4 ± 0.15
Phosphate (mg P/100 ml)					
Non injected	7.6 ± 0.1	9.3 ± 0.6	11.0 ± 0.23	9.5 ± 0.04	10.0 ± 0.31
SCT-injected	5.7 ± 0.1	6.3 ± 0.4	10.4 ± 0.18	7.9 ± 0.20	7.5 ± 0.23
"p" values	<.001	<.001	<.05	<.001	<.001

[a] Abbreviations: PTX = parathyroidectomized; TX = thyroidectomized with transplanted parathyroids; Acute TPTX = thyroparathyroidectomized and injected 1 hr later. acute Nephx = bilalaterally nephrectomized and injected 1 hr later. SCT = 25 MRC mU salmon calcitonin injected subcutaneously.

[b] Differences in plasma calcium, all groups, $p < .001$.

[c] All values given with standard error of mean.

was used in these experiments.

As noted in Table I, this level of calcitonin produced the expected drop in stable calcium and phosphate concentrations in all groups. The effect of calcitonin on ^{45}Ca disappearance from plasma agreed with that previously reported (1, 4, 5) in that the 1 hr values for the calcitonin-treated rats were only slightly reduced from the control value. For this reason only the ^{32}P data are included in this report and are summarized in Table II. The following points should be emphasized: Calcitonin produced an increased rate of loss of ^{32}P from plasma in all groups. However, there was no significant change in specific activity in any of the calcitonin-treated groups. The smaller differences produced by SCT in the two TPX groups can not be related to parathyroid hormone action on renal phosphate excretion as the effect of the hormone in nephrectomized animals was maximal. It is suggested that, in the acute TPTX group, the reduced effect may be due to excess of calcitonin released during thyroparathyroidectomy (6). The reduced effect in the 18 hr PTX group may be related to the lower starting calcium plasma level, but this conclusion needs confirmation.

Discussion. The data represented in this report add further evidence that calcitonin injection is followed by a transfer of phosphate out of plasma, rather than an inhibition of its entry into plasma which would occur if the only mechanism involved was decreased bone resorption. They also provide a clear demonstration that this action of the hormone does not require the presence of parathyroid hormone and that functional kidneys are not essential for its manifestation. The relationship of this hypophosphatemic effect to the hypocalcemic effect of calcitonin must await further study.

Summary. The effect of salmon calcitonin on the rate of disappearance of simultaneously administered ^{32}P was studied further in rats. It was demonstrated that at 1 hr postinjection the ^{32}P plasma values were significantly lower in calcitonin-injected rats than in their controls. This difference was seen in normal, thyroidectomized, nephrectomized and parathyroidectomized rats indicating that this was a direct effect of calcitonin independent of renal or parathyroid function. The data in this study confirm the earlier report that calcitonin causes a rapid movement of phosphate out of plasma.

TABLE II. Plasma ^{32}P Values 1 hr After Concurrent SCT and ^{32}P Injections.[a]

	Controls	TX 18 hr	PTX 18 hr	Acute TPTX	Acute Nephx
Plasma ^{32}P values[b]					
Noninjected	22.3 ± 1.1[c] (12)[d]	26.6 ± 2.1 (7)	37.7 ± 0.84 (12)	35.4 ± 1.5 (8)	40.1 ± 2.2 (8)
SCT-injected	16.3 ± 0.7 (12)	17.9 ± 1.0 (7)	33.2 ± 0.96 (12)	29.7 ± 1.2 (8)	28.1 ± 1.2 (8)
% of controls	73.1	67.3	88.2	84.0	70.0
p Values	<.001	<.005	<.005	<.005	<.001
Plasma ^{32}P sp act[e]					
Noninjected	3.0 ± 0.2	2.9 ± 0.16	3.4 ± 0.11	3.7 ± 0.16	4.0 ± 0.14
SCT-injected	2.9 ± 0.1	3.0 ± 0.14	3.2 ± 0.08	3.8 ± 0.09	3.8 ± 0.14

[a] Abbreviations: PTX = parathyroidectomized; TX = thyroidectomized with transplanted parathyroids; acute TPTX = thyroparathyroidectomized and injected 1 hr later; acute Nephx = bilaterally nephrectomized and injected 1 hr later; SCT = 25 MRC mU salmon calcitonin injected subcutaneously.

[b] ^{32}P plasma values are given as % injected dose × 10^{-3}.

[c] All values given with standard error of mean.

[d] Numbers in parentheses = number of rats in group.

[e] ^{32}P specific activity = % injected dose × 10^{-3}/plasma phosphate.

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