Effect of Crude Thyrocalcitonin on Calcium and Phosphorus Metabolism in Rats.* (30508)

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Hirsch and his associates have reported that thyroid extracts contain a potent hypocalcemic factor which is active in the intact and parathyroidectomized rat(1). They named this factor thyrocalcitonin, to distinguish it from calcitonin, the parathyroid hypocalcemic factor described earlier by Copp *et al*(2).

The mechanism by which thyrocalcitonin lowers serum calcium is unknown. Possible sites of removal are urinary excretion, net secretion into gut, and hard and soft tissue uptake. This study is concerned only with renal and other soft tissue sites.

Materials and methods. The rats, 8-week-old Holtzman males, were fed a normal diet; the rats used for gut analysis were fasted for 48 hours prior to the experiment. All rats were injected subcutaneously with either acid saline (pH 4) or thyroid extract; blood samples and organs were removed at intervals of 20, 30, 40 or 60 minutes after the injections. Urine, when collected, was for a 1-hour period following an oral water load (50 ml/kg) and injection. Collection of urine(3) and analysis of urinary calcium(4), urinary phosphorus(5), urinary creatinine(6), serum calcium(7), and serum phosphorus(5) were done by the methods described in the references. Two kidneys, 2 submaxillary salivary glands, 1 small intestine, and either 2 lenses (phosphorus analysis only) or 12 lenses (calcium and phosphorus analyses), were placed in platinum crucibles, dried at 105°C for 24 hours (except gut, 48 hours, and lenses, 72 hours), incinerated cautiously on a Kjeldahl digestor, and ashed for 36 hours at 600°C. The ash was dissolved in 5 ml of 5 N HCl, evaporated almost to dryness to hydrolyze any pyrophosphates, dissolved in 10 ml water, and analyzed for calcium(4) and for phosphorus(5). Crude thyrocalcitonin was prepared from bovine or hog thyroids† as follows. Thyroid tissue, 150 g, was homogenized with 1500 ml 0.1 N HCl in a Waring blendor for 2 minutes; the extract was adjusted to pH 4.0, centrifuged, and stored in the freezer.

Results. Serum and urine. Thyroid extract caused hypocalcemia, hypophosphatemia and phosphaturia one hour following injection (Table I). There were no significant effects on urinary calcium or creatinine.

Kidney, salivary glands and gut. In spite of an accompanying fall in serum calcium there were no effects on the calcium and phosphorus contents of submaxillary glands and small intestine (Table II). It is significant that the serum calcium fell to an even greater extent in the 48 fasted rats used in the gut experiment. In the kidney, thyroid extract caused a significant fall in calcium but no change in phosphorus content (Table II).

Lens. In 3 experiments thyroid extract had no effect on phosphorus content of the lens (Table III). Two experiments were done in which calcium content was determined. In the first, thyroid increased the calcium content; in the second there was no effect.

Discussion. The data did not reveal any major site of calcium removal, which could account for the fall in serum calcium following thyrocalcitonin administration. However, certain conclusions can be drawn. It was found that crude thyrocalcitonin in the rat causes: (1) a hypocalcemia in both fed and fasted rats; (2) hypophosphatemia; (3) no loss of calcium in the urine; (4) a phosphaturia; (5) a decrease in calcium content of the kidney; and (6) no significant shift of calcium from extracellular to intracellular space.

The observation that thyrocalcitonin has a hypocalcemic action confirms that made first by Hirsch and Munson(8). The fact that

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TABLE I. Effect of Crude Thyrocalcitonin on Serum and Urinary Ca and P.

Treatment	No. of rats	Serum Ca	Serum P	Urinary Ca	Urinary P	Urinary creatinine
		mg/100 ml		μg/hr		
Saline	8	10.2	—	63	430	306
Bovine thyroid	8	9.2†	—	85	799*	438
Saline	10	10.0	10.4	82	436	319
Hog thyroid	10	9.0†	8.4†	65	723*	353
Standard deviations		±.45	±.68	±28.1	±241	±114

Bovine dose: 10 ml/kg sc; hog dose: 2 ml/kg sc. The hog thyroid was a partially purified preparation kindly supplied by Dr. Philip Hirsch. Blood and urinary samples taken 1 hr after injection.

Probability levels: * <.05; † <.01.

TABLE II. Effect of Crude Bovine Thyrocalcitonin on Kidney, Salivary Gland, and Gut Ca and P.

Treatment	No. of rats	Time, min	Serum Ca, mg %	Tissue dry wt, %	Tissue Ca		Tissue P	
					mg/g wet	mg/g dry	mg/g wet	mg/g dry
				Kidney				
Saline	10	30	9.7	24.1	.076	.32	2.79	11.6
Thyroid	10	30	8.8†	24.3	.068†	.28†	2.81	11.5
Saline	10	60	10.4	24.0	.078	.32	2.05	8.5
Thyroid	10	60	8.9†	24.0	.065†	.27†	2.05	8.5
		S.D.	±.31	±.77	±.0053	±.023	±.218	±.86
				Submaxillary salivary glands				
Saline	5	20	9.2	21.3	.37	1.73	2.17	10.2
Thyroid	5	20	8.7	21.6	.41	1.88	2.41	11.2
Saline	7	40	10.4	20.8	.24	1.17	2.60	12.4
Thyroid	8	40	9.0†	21.4	.26	1.23	2.74	12.8
Saline	8	60	10.6	20.0	.24	1.19	2.59	13.0
Thyroid	7	60	8.9†	20.5	.26	1.29	2.61	12.7
		S.D.	±.60	±.90	±.041	±.171	±.315	±1.49
				Small intestine‡				
Saline	7	60	10.4	20.6	.25	1.22	1.30	6.29
Thyroid	7	60	8.1†	20.6	.22	1.08	1.32	6.34
		S.D.	±.29	±1.23	±.118	±.609	±.449	±1.948

Bovine thyroid dose: 20 ml/kg sc.

Probability levels: * <.05; † <.01. ‡ Rats fasted 48 hr.

TABLE III. Effect of Crude Thyrocalcitonin on Lens Ca and P.

Treatment	No. of rats	No. of lenses per analysis	Serum Ca, mg %	Lens Ca	Lens P
				mg/g wet	
Saline	10	2	9.8	—	.97
Bovine thyroid	10	2	9.0†	—	.93
		S.D.	±.27	—	±.10
Saline	36	12	10.1	.030	.39
Bovine thyroid	36	12	9.4†	.033*	.40
		S.D.	±.30	±.0030	±.154
Saline	36	12	9.7	.049	.84
Hog thyroid	36	12	8.7†	.045	.78
		S.D.	±.29	±.0095	±.143

Thyroid dose: 20 ml/kg sc.

Blood and lenses removed 1 hr after injection.

Probability levels: * <.05; † <.01.

thyrocalcitonin had a greater effect in the fasted rat eliminates the role of calcium absorption in the gut, a conclusion also reached by Hirsch *et al*(1). The hypophosphatemia and no loss of urinary calcium were mentioned by Kenny(9); the former response has since been confirmed by Hirsch *et al*(1). The fact that the hypocalcemic effect of thyrocalcitonin was unaccompanied by any loss of calcium in the urine indicates that the kidney is not the site of calcium removal, a conclusion reached in a different approach by Hirsch *et al*(1) but supported by no data.

The phosphaturic effect, not previously reported is interesting and undoubtedly contributes to the hypophosphatemia. It may be an artifact arising from the crude nature of the extract or it may be a secondary effect due to stimulation of parathyroid hormone release by the hypocalcemic condition. Increased glomerular filtration rate is a possible explanation, although this is unlikely in view of the lack of significant effect on urinary creatinine. The decrease in calcium content of the kidney is possibly a reflection of the general fall in extracellular calcium level. The observation may prove of clinical importance should thyrocalcitonin be used therapeutically to treat human hypercalcemia, a condition often associated with renal metastatic calcification.

No evidence was found to indicate that thyrocalcitonin caused a shift of calcium from extracellular to intracellular space, although the techniques and tissues used might have left a shift undetected. The significant shift in one lens experiment and the consistent, but not significant, trend in the salivary gland data suggest that this area should receive thorough attention with adequate techniques. This is particularly important in view of the fundamental transport mechanisms being influenced by parathyroid hormone at the mitochondrial level(10).

One possible mechanism for the hypocalcemic action of thyrocalcitonin is that it causes a net secretion into the gastrointestinal tract. Neither our data nor those of Aliapoulios *et al*(11) support this concept. The most attractive theory, suggested by Hirsch *et al*(1), is that thyrocalcitonin causes calcium deposition in bone.

Summary. Crude thyrocalcitonin when injected subcutaneously into rats caused a hypocalcemia accompanied by a hypophosphatemia and a phosphaturia. There was no increase in urinary calcium indicating that the kidney plays no role in the hypocalcemic response. The calcium content of the kidney fell, an effect which probably reflects the hypocalcemic response and could have clinical importance. The kidney phosphorus content as well as the calcium and phosphorus contents of the small intestine, submaxillary salivary glands, and the lens were not consistently affected by the drug. The data also support the conclusion that calcium transport in the gut plays no role in the hypocalcemic response.

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