Effects of Acute Administration of Porcine and Salmon Calcitonin on Urine Electrolyte Excretion in Rats (34968)

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The acute administration of porcine calcitonin (CT) to rats has been reported by several authors to elevate urine excretion of phosphorus (13, 15, 20, 21), calcium (16) and sodium (20). In contrast, Pechet *et al.* (19), and Aliapoulis and Munson (1) reported that calcitonin reduced urinary calcium, phosphorus, and magnesium in the rat. Smith *et al.* (14) found that porcine calcitonin, including homogeneous material, increased the excretion of water, sodium, and potassium in the rabbit, while Clark *et al.* (10), and Clark and Kenny (9) found no effect of crude porcine calcitonin on urinary calcium, phosphorus, sodium, or magnesium in the dog. In man, Ardaillou and co-workers reported that intravenous infusions of porcine calcitonin produced increased excretion rates of phosphate, calcium (2, 3), sodium, and chloride (4, 5), with no change in clearance of inulin. They concluded that calcitonin acts directly on the proximal kidney tubule to inhibit sodium and calcium reabsorption (6) independently. Similar results in man with porcine calcitonin have also been reported by Bijvoet *et al.* (7), Singer *et al.* (22, 23), Martin and Melick (14), and Cochran *et al.* (11).

The purpose of the present study was to investigate the comparative effects upon urine electrolytes of the acute administration of porcine and salmon calcitonin in rats.

Materials and Methods. Calcitonin preparations utilized for these experiments were: (a) porcine calcitonin, 60 MRC units/mg solids[1] (Armour Lot K423-082); or (b) salmon calcitonin, 1500 MRC units/mg solids[1] (Armour Lot K423-084), extracted from salmon ultimobranchial tissue. All preparations were administered subcutaneously in 16% gelatin vehicle (Armour Lot K281-282) in a volume of 0.1 ml/100 g.

Effects of porcine and salmon CT on serum and urine electrolytes were measured over a 1–5-hr period following CT injection. Male Holtzman rats weighing 220–250 g were maintained in metabolism cages, 2–3 rats/cage, following an overnight fast. Food and water were not allowed during the experiment. In all experiments except one, an intraperitoneal saline load of 3.3 ml/100 g of body weight was administered simultaneously with calcitonin injection.

In experiments in which serum electrolytes were measured at the end of the collection period, the rats were anesthetized and blood was drawn from the abdominal aorta.

Serum and urine electrolytes were measured by atomic absorption spectrometry, phosphate was determined by the method of Fiske and Subbarow (12), and creatinine by the method of Bonsnes and Taussky (8).

Experiments and Results. Table I shows effects of single sc injections of 0.2 and 2 MRC units/100 g of pork or salmon CT on urine sodium and potassium excretion in rats during a 5-hr collection period. While porcine calcitonin, at the doses given, did not significantly influence excretion of sodium or potassium, a slight decrease in the former together with a slight increase in the latter resulted in a significant depression in the sodium:potassium ratio. In addition, urine volume was significantly decreased at the higher dose (2U/100 g) of porcine calcitonin. In contrast, 2 U/100 g, but not 0.2 U/100 g of salmon CT elevated urine volume 3-fold, and

[1] Unitage is based on the 1-hr hypocalcemic activity in the rat of the test preparation administered subcutaneously, compared to the Medical Research Council (MRC) porcine calcitonin Standard B.

TABLE I. Effects of Porcine and Salmon CT on Urine Electrolytes in the Saline-Loaded Rat.[e]

Treatment	Dose (U/100 g)	Urine vol (ml)[a]	Total (mg)[a] Na	Total (mg)[a] K	Na/K
Control	0	2.2 ± 0.3	9.3 ± 1.1	5.6 ± 1.2	1.73 ± 0.26
Porcine CT	0.2	1.9 ± 1.0	6.5 ± 2.3	7.4 ± 2.1	0.91 ± 0.29[c]
	2.0	1.2 ± 0.5[b]	7.3 ± 3.1	6.2 ± 2.4	1.16 ± 0.09[c]
Salmon CT	0.2	2.2 ± 0.8	10.9 ± 3.1	7.0 ± 1.0	1.53 ± 0.28
	2.0	6.7 ± 1.6[c]	34.0 ± 5.4[d]	4.9 ± 0.3	6.92 ± 1.19[d]

[a] Per rat.
[b] $p < .05$.
[c] $p < .01$.
[d] $p < .001$.
[e] $N = 4$ replicates of 2 rats/group.

total urinary sodium 3.5-fold, without influencing potassium excretion, resulting in significant elevation of the sodium:potassium ratio.

Table II shows effects of two doses of salmon calcitonin, and a high dose of porcine calcitonin on urine electrolyte excretion in the rat. Both 0.5 and 2.0 U/100 g of salmon CT produced significant elevations in urine volume, sodium, potassium, and calcium; the most dramatic effect being on sodium excretion, as indicated by a significant increase in the sodium:potassium ratio. As in the first experiment (Table I) a somewhat greater potassium excretion occurred with the lower dose of salmon calcitonin compared to the higher dose. Porcine calcitonin, at a dose of 4U/100 g, produced a significant increase in urine sodium and potassium excretion, although lower doses were ineffective (Table I). However, the sodium:potassium ratio was not significantly changed by porcine calcitonin.

Table III shows effects of graded doses of salmon CT on urine electrolytes in the 5-hr saline-loaded rat assay. As in the first experiment (Table I) 0.2 U/100 g of salmon CT did not significantly affect urine sodium, potassium, or calcium excretion during the experimental period. However, doses of 0.5 to 2.0 U/100 g produced dose-related increases in urine volume and sodium excretion. Only slight increases in potassium excretion were noted, resulting in significant elevations in the sodium:potassium ratios. Urine calciums were increased significantly at the 2 and 4 U/100 g dosages.

TABLE II. Effects of Salmon and Porcine CT on Urine Electrolytes in the Saline-Loaded Rat.[e]

Treatment	Dose (U/100 g)	Urine vol (ml)[a]	Total (mg)[a] Na	Total (mg)[a] K	Na/K	Total Ca (mg)[a]
Control	0	1.5 ± 0.3	6.1 ± 1.0	2.9 ± 0.4	2.12 ± 0.28	0.03 ± 0.01
Salmon CT	0.5	3.5 ± 0.6[d]	17.8 ± 5.0[c]	5.3 ± 1.4[c]	3.33 ± 0.18[d]	0.045 ± 0.01[b]
	2.0	5.0 ± 0.2[d]	26.6 ± 2.4[d]	3.8 ± 0.4[b]	7.11 ± 1.1[d]	0.06 ± 0.01[d]
Porcine CT	4.0	2.0 ± 0.6	11.4 ± 2.5[c]	6.2 ± 2.0[b]	1.95 ± 0.65	0.035 ± 0.01

[a] Per rat.
[b] $p < .05$.
[c] $p < .01$.
[d] $p < .001$.
[e] $N = 4$ replicates of 2 rats/group.

TABLE III. Effects of Graded Doses of Salmon CT on Urine Electrolytes in the Saline-Loaded Rat.[e]

Treatment	Dose (U/100 g)	Urine vol (ml)[a]	Total (mg)[a] Na	Total (mg)[a] K	Na/K	Total Ca (mg)[a]
Control	0	2.0 ± 0.4	9.9 ± 2.5	4.2 ± 1.3	2.35 ± 0.21	0.05 ± 0.02
Salmon CT	0.2	2.1 ± 0.2	9.0 ± 1.1	5.2 ± 0.5	1.71 ± 0.10[c]	0.04 ± 0.01
	0.5	4.0 ± 0.6[c]	17.3 ± 2.1[c]	6.0 ± 0.4[b]	2.88 ± 0.50	0.06 ± 0.01
	1.0	4.4 ± 1.2[b]	24.0 ± 4.5[c]	4.4 ± 0.9	5.77 ± 2.11[b]	0.06 ± 0.01
	2.0	6.8 ± 0.9[d]	35.5 ± 4.6[d]	4.8 ± 0.6	7.49 ± 1.22[c]	0.09 ± 0.01[b]
	4.0	5.5 ± 0.7[c]	33.9 ± 1.9[d]	4.7 ± 0.8	7.28 ± 0.78[d]	0.09 ± 0.02[b]

[a] Per rat.
[b] $p < .05$.
[c] $p < .01$.
[d] $p < .001$.
[e] $N = 3$ replicates of 2 rats/group, except doses of 0.5 and 1.0 U/100 g, which contained 4 replicates of 2 rats/group.

Tables IV and V show urine and blood data, respectively, from an experiment in which groups of rats were treated with 2 U/100 g of salmon CT in 16% gelatin vehicle without saline loading. Urines were collected from separate groups of rats (4 cages/group; 3 rats/cage) at the end of 1, 3, and 5-hr post-treatment, at which times blood samples were drawn from the abdominal aorta and the animals were sacrificed.

Salmon CT caused no significant changes in any of the urine parameters measured during the first hour following treatment. In the 3-hr group, salmon CT caused a doubling of urine volume and a 3-fold increase in urine sodium excretion, with no significant change in potassium excretion, resulting in an elevation of the sodium:potassium ratio (Table IV). In addition, salmon CT produced significant increases in urine phosphorus and pH, and a decrease in magnesium excretion. Similar results were observed in the 5-hr group; there were also significant increases in urine potassium, chloride, calcium, and creatinine.

Salmon CT treatment produced hypocalcemia and hypophosphatemia at 1, 3, and 5 hr post-treatment (Table V). Serum magnesium was elevated 1 hr after treatment, but no significant effects were observed on this parameter at later time intervals. Serum potassium was slightly but significantly increased 5 hr post-treatment. No significant changes in hematocrit were observed.

Discussion. Results of these experiments indicate that salmon CT produces, acutely, a marked water, sodium, potassium, chloride, phosphorus, and calcium diuresis in the normal rat. In addition, salmon CT produces significant retention of magnesium and an elevation of urine pH. These effects appear to be dose related and, for the parameters measured, occur in both nonloaded and saline-loaded rats. Preliminary data from this laboratory indicate that salmon CT also increases sodium excretion in the dog.

In the rat, salmon CT elevates, acutely, sodium excretion to a greater extent than potassium or calcium. The hormone produces hypocalcemia and hypophosphatemia, but does not significantly influence serum sodium, potassium, or magnesium levels. Although a significant elevation of creatinine excretion occurs, the primary effect of salmon CT on sodium, phosphorus, and calcium excretion probably involves decreases in the renal tubular reabsorption of these electrolytes (calcium and phosphorus diuresis occur concomitantly with highly significant hypocalcemia and hypophosphatemia). It has been suggested by Nordin and Peacock (17) and Cochran and co-workers (11) the acute serum calcium lowering with calcitonin in hypercalcemic patients is largely due to a de-

TABLE IV. Effects of Salmon CT on Urine Electrolytes in the Nonloaded Rat.[e]

	1 hr		3 hr		5 hr	
	Control	CT	Control	CT	Control	CT
Urine vol (ml)[a]	0.5 ± 0.2	0.4 ± 0.2	0.6 ± 0.1	1.1 ± 0.1[d]	0.6 ± 0.1	2.1 ± 0.6[c]
Total (mg)[a] Na	1.6 ± 1.0	1.9 ± 0.5	2.5 ± 1.5	7.9 ± 0.4[d]	1.9 ± 0.3	13.8 ± 2.7[d]
K	4.8 ± 3.1	4.2 ± 2.0	5.1 ± 3.8	7.5 ± 3.5	2.4 ± 0.4	3.9 ± 0.7[c]
Na/K	0.33 ± 0.02	0.50 ± 0.15	0.55 ± 0.11	1.22 ± 0.47[b]	0.8 ± 0.18	3.6 ± 1.0[c]
Total (mg)[a] Ca	0.007 ± 0.003	0.01 ± 0.003	0.013 ± 0.003	0.017 ± 0.003	0.01 ± 0.003	0.02 ± 0.007[c]
Mg	0.09 ± 0.02	0.06 ± 0.04	0.16 ± 0.02	0.03 ± 0.01[d]	0.18 ± 0.08	0.05 ± 0.01[b]
P	2.1 ± 0.9	1.8 ± 0.7	2.6 ± 0.5	3.8 ± 0.7[b]	2.4 ± 0.5	4.5 ± 0.2[d]
Cl	2.5 ± 1.5	1.8 ± 0.5	4.1 ± 2.6	6.9 ± 0.6	3.0 ± 0.5	11.6 ± 2.5[d]
Creatinine	1.6 ± 0.5	1.3 ± 0.4	2.4 ± 0.6	3.0 ± 0.5	2.4 ± 0.6	3.7 ± 0.6[b]
pH	6.29 ± 0.30	6.63 ± 0.05	5.92 ± 0.27	7.60 ± 0.07[d]	5.84 ± 0.12	7.76 ± 0.15[d]

[a] Per rat.
[b] $p < .05$.
[c] $p < .01$.
[d] $p < .001$.
[e] $N = 4$ replicates of 3 rats/cage; dosage: 2 U/100 g.

TABLE V. Effects of Salmon CT on Blood Electrolytes in the Nonloaded Rat.[c]

	1 hr		3 hr		5 hr	
	Control	CT	Control	CT	Control	CT
(mg %)						
Na	387 ± 32	382 ± 48	374 ± 28	354 ± 31	334 ± 30	361 ± 19
K	31.7 ± 4.2	27.7 ± 4.4	28.7 ± 3.2	27.5 ± 2.8	26.4 ± 4.1	30.0 ± 2.4[a]
Ca	8.82 ± 0.31	7.52 ± 0.27[b]	9.05 ± 0.43	6.45 ± 0.32[b]	9.46 ± 0.58	6.12 ± 0.57[b]
Mg	2.39 ± 0.12	2.63 ± 0.09[b]	2.45 ± 0.34	2.65 ± 0.17	2.27 ± 0.15	2.31 ± 0.18
P	9.12 ± 0.74	7.13 ± 0.69[b]	9.68 ± 0.84	6.49 ± 0.59[b]	9.86 ± 0.62	6.35 ± 0.77[b]
pH	7.92 ± 0.07	7.88 ± 0.07	7.90 ± 0.08	7.90 ± 0.06	7.92 ± 0.06	7.96 ± 0.06
Hematocrit (%)	43.6 ± 1.8	44.6 ± 1.4	46.9 ± 1.7	47.2 ± 2.3	48.8 ± 2.3	48.8 ± 1.2

[a] $p < .05$.
[b] $p < .001$.
[c] $N = 4$ replicates of 3 rats/cage; dosage: 2 U/100 g.

crease in tubular reabsorption of calcium, and that the kidneys play a major role in calcium homeostasis (18).

In contrast to the effects on other ions, a marked decrease in urinary magnesium, with no change in serum magnesium, occurs following salmon CT administration to the rat. A decrease in urinary magnesium in rats following porcine CT has been reported by Pechet *et al.* (19), and by Aliapoulis and Munson (1).

It should be emphasized that the minimally effective natriuretic dosage of salmon CT in the saline-loaded rat is approximately 100 times that required to produce hypocalcemia (in young, fasted rats). Even so, a doubling of urinary sodium occurs with a dose of 0.14 μg/100 g (pure hormone equivalent based on a specific activity of 3500 MRC U/mg for synthetic salmon CT (25)) and a maximal effect, involving a 3.5 to 7-fold increase of urinary sodium, occurs with a dose of 0.56 μg/100 g, indicating that salmon calcitonin may be one of the most potent natriuretic agents known.

On a unitage basis (for hypocalcemic effect), porcine CT required an 8-fold greater dose than salmon CT to produce significant natriuresis in the rat. However, porcine CT has been reported to enhance sodium excretion in man (4, 5, 7, 14, 22, 23) at much lower doses (on a body wt basis) than are required in the rat.

The results reported here with salmon CT, and the numerous literature reports with porcine CT point to the possible physiological (and/or pathological) participation of endogenous calcitonin in the regulation of sodium excretion (and other electrolytes) by the kidney. Further studies involving endogenous calcitonin or calcitonin derived from the test species itself will be required before definitive conclusions can be drawn. It will also be of interest to determine the urinary electrolyte effects of exogenous human calcitonin in man.

Summary. Acute sc administration of natural salmon CT to fasted male rats, at a dosage of 0.5 MRC U/100 g or greater, produced an alkaline diuresis, natriuresis, hypercalciuria, hyperphosphaturia, and hypomagnesuria. Urine potassium and creatinine were less markedly, but significantly, elevated. During the 5-hr observation period, hypocalcemia and hypophosphatemia were apparent, with no consistent changes in blood sodium, potassium, magnesium, or pH. Similar results were obtained in rats given an intraperitoneal saline load. In the same assay system, porcine CT, at doses up to 2.0 U/kg sc, produced similar blood changes, but did not influence urine parameters.

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