Requirement of the Adrenal for Certain Urine Electrolyte Effects of Salmon Calcitonin in Rats[1] (35744)

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(Introduced by R. J. Schlueter)

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We have recently reported the effects of acute administration of salmon calcitonin (CT) on urine electrolyte excretion in rats (1). Doses of 0.5 MRC U/100 g or greater were found to produce an alkaline diuresis, natriuresis, chloruresis, hypercalciuria, hyperphosphaturia, and hypomagnesuria. Urine potassium and creatinine were less markedly but significantly elevated. In contrast, porcine CT, at doses up to 2.0 U/100 g, did not significantly influence urine parameters. In the present communication it is shown that the effect of salmon CT on the urinary electrolyte pattern in the intact rat requires the presence of the adrenal gland.

Materials and Methods. Salmon calcitonin was extracted from ultimobranchial glands (Armour Lot K423-121) and contained 1500 MRC units/mg solids[2]. This preparation was administered sc in all experiments in a vehicle of 16% gelatin (Armour Lot K423-117 or 281-282) in a volume of 0.1 ml/100 g.

Furosemide and triamterene were suspended in steroid-suspending vehicle (SV17847,[3] Armour Lot K188-063) and administered *per os* in a volume of 0.5 ml/100 g. Desoxycorticosterone acetate (DOCA), hydrocortisone alcohol (HCT), epinephrine, and aldosterone were suspended in SV17874 vehicle and administered sc in a volume of 0.1 ml/100 g.

Effects of drug treatment on urine electrolytes were measured over a 5- or 7-hr period after treatment. Male Holtzman rats weighing 200–250 g were maintained in metabolism cages, three rats/cage. In experiments involving intact rats, food and water were allowed *ad libitum* prior to, but not during, the collection period except in one series, as noted, in which the animals were fasted 16 hr prior to urine collection. An intraperitoneal saline load of 3.3 cc/100 g was administered immediately prior to urine collection in one series of intact rats, as noted.

In experiments involving adrenalectomized (adrenx) rats, bilateral adrenalectomy was performed 3–7 days prior to the day of the experiment, during which time food and 1% NaCl drinking water were provided *ad libitum*. Twenty-four hours prior to urine collection 1% NaCl was replaced with distilled water. Food and water were allowed prior to, but not during, the collection period. In all adrenx experiments an intraperitoneal saline load of 3.3 cc/100 g was administered immediately prior to initiation of the urine collection period. Urine electrolytes were measured by atomic absorption spectrometry; phosphate was determined by the method of Fiske and SubbaRow (2), and creatinine by the method of Bonsnes and Taussky (3).

Experiments and Results. In Table I are shown the effects of single sc injections of varying doses of salmon CT on urine electrolyte excretion in intact rats. A comparison was made of CT effects in nonfasted rats with or without saline loading vs 16-hr fasted rats without saline loading. These data may be compared to data we recently reported utilizing fasted, saline-loaded rats (1). Qualitatively similar effects of CT were observed throughout, including increases in the volume

[1] This study was presented in part at the 55th Annual Meeting of the Federation of American Societies for Experimental Biology, April 14, 1971.

[2] 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.

[3] Each milliliter contains sodium chloride, 9 mg; sodium carboxymethylcellulose 7 LP, 5 mg; Polysorbate 80 USP, 0.004 ml; benzyl alcohol, 0.009 ml in water for injection.

TABLE I. Effect of Salmon Calcitonin on Urine Electrolytes in the Intact Rat.[a]

Treatment	Dose (U/100 g)	Urine vol (ml)[b]	Total (mg)[b]		Na/K	Total Ca (mg)[b]	Total Mg (mg)[b]
			Na	K			
Nonfasted, saline-loaded							
Control	0	3.8 ± 0.6	7.5 ± 1.3	15.6 ± 0.6	0.48 ± 0.09	0.15 ± 0.05	0.88 ± 0.26
CT	0.2	2.6 ± 0.5	11.4 ± 1.8[c]	17.6 ± 2.0	0.65 ± 0.04[c]	0.12 ± 0.02	0.32 ± 0.05[d]
	0.5	5.9 ± 1.5[d]	22.5 ± 5.7[d]	17.9 ± 2.6	1.24 ± 0.19[e]	0.20 ± 0.04	0.62 ± 0.14
	1.0	6.9 ± 1.1[e]	27.0 ± 0.6[e]	16.6 ± 1.4	1.63 ± 0.15[e]	0.24 ± 0.04[e]	0.71 ± 0.17
Nonfasted, Nonloaded							
Control	0	1.1 ± 0.4	3.5 ± 1.4	12.6 ± 3.1	0.27 ± 0.05	0.08 ± 0.03	0.42 ± 0.06
CT	0.1	1.3 ± 0.2	5.2 ± 1.0	14.8 ± 2.6	0.35 ± 0.04[c]	0.10 ± 0.02	0.54 ± 0.22
	0.5	3.2 ± 0.7[d]	11.0 ± 3.4[d]	22.4 ± 3.3[d]	0.48 ± 0.12[c]	0.12 ± 0.04	0.40 ± 0.20
	1.0	4.3 ± 0.8[e]	17.4 ± 1.9[e]	21.7 ± 7.6	0.86 ± 0.26[d]	0.11 ± 0.02	0.41 ± 0.14
	2.0	4.1 ± 0.9[e]	20.9 ± 1.6[e]	18.9 ± 1.9[c]	1.12 ± 0.19[e]	0.11 ± 0.02	0.34 ± 0.12
Fasted, Nonloaded							
Control	0	0.9 ± 0.2	1.9 ± 0.4	2.8 ± 0.7	0.70 ± 0.17	0.03 ± 0.01	0.35 ± 0.13
CT	0.2	1.0 ± 0.4	2.9 ± 1.3	5.3 ± 0.9[d]	0.53 ± 0.18	0.04 ± 0.01	0.16 ± 0.06[c]
	0.5	1.4 ± 0.1[d]	7.0 ± 1.7[d]	5.4 ± 0.9[d]	1.31 ± 0.39[c]	0.05 ± 0.01	0.15 ± 0.02[c]
	1.0	2.4 ± 0.7[d]	12.6 ± 2.9[e]	6.0 ± 0.6[e]	2.12 ± 0.51[d]	0.07 ± 0.02[d]	0.19 ± 0.05

[a] N = Four replicates of three rats/group. The urine collection period was 5 hr.
[b] Mean per rat ± SD of mean.
[c] $p < .05$.
[d] $p < .01$.
[e] $p < .001$.

TABLE II. Effect of Salmon Calcitonin and DOCA on Urine Electrolytes in the Adrenalectomized Rat.[a]

Treatment	Urine vol (ml)[b]	Total (mg)[b] Na	K	Na/K
Control	3.5 ± 0.6	11.1 ± 2.6	23.2 ± 2.3	0.48 ± 0.11
CT (2 U/100 g)	1.8 ± 0.4[c]	6.7 ± 0.5[d]	14.9 ± 0.7[e]	0.45 ± 0.04
DOCA (0.01 mg/100 g)	3.4 ± 0.4	5.7 ± 1.2[c]	25.1 ± 1.7	0.22 ± 0.03[e]
Salmon CT + DOCA (2 U/100 g + 0.01 mg/100 g)	1.4 ± 0.1[e]	4.9 ± 0.4[c]	13.1 ± 1.2[e]	0.37 ± 0.02

[a] N = Four replicates of three rats/group. Rats were nonfasted and saline-loaded prior to treatment and urine collection period of 7 hr.
[b] Mean per rat ± SD of mean.
[c] $p < .01$.
[d] $p < .02$.
[e] $p < .001$.

of urine excreted, natriuresis, slight to significant increases in potassium and calcium excretion, and hyperphosphaturia (1). However, rats which were not administered a saline load generally showed larger relative increases in potassium excretion after CT treatment than did rats which were saline loaded (Table I and Ref. 1). Also, consistent reductions in magnesium excretion occurred only in rats fasted prior to urine collection. However, recent experiments not reported herein have shown consistent and significant reductions in urine magnesium after salmon CT administration to nonfasted, saline-loaded, intact rats.

Table II shows effects in adrenalectomized, saline-loaded rats of a dose of salmon CT which produced maximal diuresis and natriuresis in intact rats (1). In adrenx rats, salmon CT produced significant decreases in urine volume and in sodium and potassium excretion with no change in the sodium:potassium ratio, in contrast to results in intact rats.

Administration of .01 mg/100 g sc of DOCA to adrenx rats did not affect urine volume or potassium excretion, but significantly decreased the excretion of sodium, producing a decrease in the sodium:potassium ratio. A combination of salmon CT and DOCA resulted in urine electrolyte shifts similar to those observed with salmon CT alone.

In view of the above results, the effects of two known diuretic drugs, furosemide and triamterene, each of which acts independently of the adrenal gland (5, 6) were compared to salmon CT in adrenx rats (Table III). Confirming results in Table II, salmon CT in adrenx, saline-loaded rats produced a decrease in urine volume and in sodium and potassium excretion during the 5-hr period after treatment. In contrast, furosemide (3.0 mg/100 g *per os*) produced a significant diuresis, natriuresis, and kaluresis, and an increase in the sodium:potassium ratio. Triamterene (1.0 mg/100 g *per os*) did not increase urine volume, but significantly increased urine sodium output and decreased potassium output, resulting in a highly significant elevation in the sodium:potassium ratio. Thus, although the presence of the adrenal gland in the rat was found to be a prerequisite for the natriuretic effect of salmon CT, such was not the case with furosemide and triamterene.

A preliminary experiment was then performed to attempt to determine what factor(s) from the adrenal gland is necessary for the observed effect of salmon CT on urine electrolyte excretion in intact rats. Treatment of adrenx, saline-loaded rats with salmon CT

TABLE III. Effect of Salmon Calcitonin, Furosemide, and Triamterene on Urine Electrolytes in the Adrenalectomized Rat.[a]

Treatment	Urine vol (ml)[b]	Total (mg)[b] Na	Total (mg)[b] K	Na/K
Control	3.4 ± 0.4	9.3 ± 1.1	20.5 ± 1.0	0.46 ± 0.02
CT (2 U/100 g)	1.7 ± 0.8[c]	6.8 ± 3.7	12.1 ± 4.1[c]	0.53 ± 0.17
Furosemide (3 mg/100 g)	11.1 ± 1.9[d]	26.2 ± 4.3[d]	27.4 ± 2.4[c]	0.96 ± 0.19[c]
Triamterene (1 mg/100 g)	3.6 ± 0.9	23.7 ± 6.8[c]	7.7 ± 4.5[c]	3.44 ± 0.88[d]

[a] N = Four replicates of three rats/group. Rats were nonfasted and saline-loaded prior to treatment and urine collection period of 5 hr.
[b] Mean per rat ± SD of mean.
[c] $p < .01$.
[d] $p < .001$.

alone resulted (Table IV) in a 5-hr urine excretory pattern similar to that observed in previous experiments (Tables II and III). Pretreatment of adrenx rats with arbitrarily selected doses of aldosterone and epinephrine 1 hr prior to salmon CT administration did not restore the pattern of urine electrolyte excretion observed in intact rats, but aldosterone did prevent the potassium retention induced by salmon CT alone, resulting in a significant decline in the sodium:potassium ratio. Hydrocortisone alcohol (.25 mg/100 g sc), administered to adrenx rats 1 hr prior to treatment with 2.0 U/100 g sc of salmon CT and saline loading, completely prevented the decline in urine volume and in sodium and potassium excretion noted with salmon CT alone, but did not restore the diuretic–natriuretic effect of salmon CT observed in intact rats.

Additional experiments were performed in which HCT was administered to adrenx rats once daily for 3 days after adrenx, in addition to HCT treatment 1 hr prior to salmon

TABLE IV. Effect of Salmon Calcitonin, Epinephrine, Aldosterone and Hydrocortisone on Urine Electrolytes in the Adrenalectomized Rat.[a]

Treatment	Urine vol (ml)[b]	Na	K	Na/K
Control	3.1 ± 0.4	8.8 ± 3.0	19.8 ± 1.6	0.45 ± 0.07
CT (2 U/100 g)	1.2 ± 0.5[c]	4.4 ± 1.3[c]	11.3 ± 3.6[d]	0.39 ± 0.03
CT + epinephrine (.04 mg/100 g)	1.1 ± 0.6[c]	3.5 ± 2.1[d]	9.3 ± 5.8[d]	0.38 ± 0.01
CT + aldosterone (.005 mg/100 g)	1.6 ± 0.2[c]	3.5 ± 2.6[e]	17.4 ± 2.2	0.19 ± 0.12[e]
CT + HCT (.25 mg/100 g)	3.1 ± 0.4	9.1 ± 1.9	20.1 ± 2.2	0.45 ± 0.08

[a] N = Three replicates of three rats/group. HCT, epinephrine, and aldosterone were given 1 hr prior to CT, and urine collection period was 5 hr. Rats were nonfasted and saline-loaded prior to treatment.
[b] Mean per rat ± SD of mean.
[c] $p < .001$.
[d] $p < .02$.
[e] $p < .05$.

TABLE V. Effects of Salmon Calcitonin and Hydrocortisone on Urine Electrolytes in the Adrenalectomized rat.[a]

Treatment	Urine vol (ml)[b]	Total (mg)[b] Na	Total (mg)[b] K	Na/K	Total Ca (mg)[b]	Total Mg (mg)[b]	Total P (mg)[b]	Total Creatinine (mg)[b]	Serum Ca (mg/100 ml)
Control	3.4 ± 1.0	7.5 ± 2.3	18.4 ± 4.3	0.41 ± 0.10	0.060 ± 0.007	0.89 ± 0.22	1.5 ± 0.3	5.8 ± 1.0	10.06 ± 0.65
CT (2 U/100 g)	1.6 ± 0.6[c]	4.5 ± 0.8	13.0 ± 3.2	0.36 ± 0.08	0.027 ± 0.003[d]	0.12 ± 0.08[c]	2.0 ± 0.4	4.5 ± 1.0	7.29 ± 0.27[e]
CT + HCT (.25 mg/100 g)	6.3 ± 2.3	17.7 ± 6.1[c]	26.3 ± 3.7[c]	0.70 ± 0.37	0.097 ± 0.053	0.21 ± 0.05[e]	8.4 ± 0.7[e]	7.1 ± 0.6	7.73 ± 0.18[e]
HCT (.25 mg/100 g)	6.0 ± 0.7[d]	8.6 ± 1.8	32.9 ± 1.9[e]	0.26 ± 0.05[c]	0.137 ± 0.080	1.31 ± 0.14[c]	7.7 ± 0.8[e]	6.7 ± 0.2	10.30 ± 0.24[d]
Control	3.8 ± 0.6	5.7 ± 1.1	16.4 ± 3.2	0.35 ± 0.01	0.062 ± 0.020	0.51 ± 0.21	2.1 ± 0.6	4.9 ± 0.3	
CT (2 U/100 g)	1.3 ± 0.1[e]	2.7 ± 0.6[d]	10.3 ± 2.5[c]	0.28 ± 0.14	0.031 ± 0.004	0.14 ± 0.07[c]	2.2 ± 0.4	3.9 ± 0.2[d]	
CT + HCT (.25 mg/100 g)	4.4 ± 1.0	11.5 ± 2.1[d]	17.5 ± 1.7	0.65 ± 0.06[e]	0.051 ± 0.017	0.09 ± 0.06[d]	5.4 ± 1.2[d]	4.9 ± 0.7	
HCT (.25 mg/100 g)	5.3 ± 1.0[c]	4.8 ± 1.5	21.6 ± 1.1[c]	0.22 ± 0.07[d]	0.103 ± 0.035[c]	0.67 ± 0.24	5.4 ± 0.6[e]	5.3 ± 0.2	

[a] N = Four replicates of three rats/group. HCT given for 3 days prior to experiment and 1 hr prior to CT, and urine collection period of 5 hr. Rats were nonfasted and saline-loaded prior to treatment.

[b] Mean per rat ± SD of mean.

[c] $p < .05$.

[d] $p < .01$.

[e] $p < .001$.

CT and urine collection on day 4. In Table V are shown results of two replicate experiments of this design. Pretreatment of adrenx rats for 3 days with HCT alone did not influence sodium excretion on day 4, but partially restored the natriuretic effect of salmon CT observed in intact rats. However, the magnitude of natriuresis in adrenx rats given HCT and CT was not so great (2-fold) as that in intact rats treated with CT alone (4- to 6-fold). Although pretreatment of adrenx rats with HCT alone did not affect sodium excretion, HCT significantly increased urine volume and potassium excretion compared to controls. Simultaneous HCT and salmon CT did not further increase urine volume but did reduce the kaluresis induced by HCT. Urine calcium appeared to be elevated by HCT and was reduced by simultaneous HCT and CT, while CT alone produced a significant decline in urine calcium in adrenx rats. Urine magnesium was increased by HCT alone, but was significantly decreased by CT or HCT plus CT. Urine magnesium was also decreased by CT in intact fasted rats, with or without saline loading (Table I, Ref. 1, and unpublished results). Urinary output of inorganic phosphate in adrenx rats was not increased by salmon CT, in contrast to effects observed in intact rats (1). Pretreatment of adrenx rats with HCT increased urine phosphate excretion compared to control rats, and a combination of HCT and salmon CT resulted in urinary phosphate excretion similar to that with HCT alone.

Urinary creatinine excretion was slightly but not significantly decreased by salmon CT in adrenx rats, while a slight but significant increase was observed in intact rats (1). The observed decline in creatinine excretion with salmon CT in adrenx rats, indicating a possible decrease in GFR, however, was not of sufficient magnitude to account for the decrements in urine electrolyte excretion induced by CT in adrenx rats. HCT did not significantly modify creatinine excretion in adrenx rats, nor did a combination of HCT and CT.

Serum calcium was depressed to an equal extent in adrenx rats administered CT, or CT plus HCT, while HCT alone increased serum calcium slightly but significantly (Table V).

Discussion. We have previously reported that acute administration of salmon CT to intact rats results in an alkaline diuresis, with concomitant elevations in urinary sodium, potassium, chloride, phosphorus, and calcium, and reduction in urinary magnesium (1). The results of the present experiments indicate that the relative magnitude of urine electrolyte changes induced by salmon CT in the intact rat are dependent upon such experimental conditions as the use of fasted vs fed animals, or saline-loaded vs nonloaded animals. In addition, it is apparent that several of the urine electrolyte shifts produced by salmon CT are dependent upon the presence of an intact adrenal gland. Dependence upon the adrenal for efficacy of a non-aldosterone antagonist-type diuretic is not without precedence (4).

Specifically, the presence of the adrenal gland was required for the enhanced excretion by salmon CT of water, sodium, and phosphorus. (The observed depression in creatinine excretion of approximately 20% is not sufficient to account for the changes in electrolyte excretion.) However, replacement therapy with a glucocorticoid only partially restored the capability of CT to induce natriuresis, and did not duplicate effects observed in the intact rat. Gaunt *et al.* (4) were unable to fully restore to the adrenx rat, by pretreatment with hydrocortisone, the pattern of electrolyte excretion induced in the intact rat by SU-15049. However, hydrocortisone pretreatment in adrenx rats more completely restored the natriuretic effect of SU-15049 (4) than was observed in the present experiments with salmon CT.

Effects of salmon CT on potassium and calcium excretion were rather variable but usually upward in intact rats, while consistent depressions in potassium and calcium excretion were seen in adrenx rats. HCT, at the dosage given, produced kaluresis and hypercalciuria in adrenx rats which were partially or completely inhibited by concomitant CT treatment. CT decreased magnesium excretion in both intact and adrenx rats under certain experimental conditions, in addition to completely inhibiting the elevated magnesium excretion produced by HCT in adrenx rats. However, CT did not modify the

diuresis and phosphaturia induced by HCT in adrenx rats.

Salmon CT does not appear to act as a mineral-corticoid antagonist in adrenx rats, since it did not prevent the sodium retention induced by DOCA or aldosterone. However, aldosterone, but not DOCA, was effective in preventing the retention of potassium induced by CT in adrenx rats.

Much study has been devoted to the well-known natriuretic effects of furosemide (5) and triamterene (6), and their proposed mechanisms of action. We have found both of these agents to exert essentially full activity in adrenx rats compared to intact rats (unpublished results). Thus, it would appear that salmon CT must exert its effects on urine electrolytes by a mechanism different from each of these agents, and, unlike these agents, is dependent upon the presence of adrenal glucocorticoid secretion.

The ability of HCT pretreatment to partially restore the natriuretic effect of salmon CT may represent another manifestation of the well-documented permissive effect of glucocorticoids as proposed by Ingle (7). A combination of adrenal hormones may be required to fully restore the excretory pattern produced by salmon CT in the intact rat, but this is only speculative.

If CT acted by a substantially different mechanism than furosemide or triamterene, one might expect a greater total urine electrolyte effect from a combination of CT and either of these agents than with either of these agents alone. However, for unknown reasons, a combination of CT and furosemide or CT and triamterene in the intact saline-loaded rat, resulted in a degree of natriuresis, as well as water and potassium excretion, no greater than that produced by CT alone. In contrast, a combination of furosemide and triamterene produced greater natriuresis than either agent alone (unpublished results).

Summary. Salmon calcitonin, a potent diuretic, natriuretic, phosphaturic agent in the intact rat, was found to be inactive in this regard in the adrenalectomized rat while maintaining full hypocalcemic activity. Pretreatment of adrenalectomized rats with hydrocortisone alcohol partially restored the natriuretic effect of salmon calcitonin, but did not duplicate effects observed in the intact rat. Other adrenal hormones were independently ineffective in restoring the urine electrolyte effects of salmon CT in the adrenx rat under the experimental conditions utilized.

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