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Modulation by calcitonin of magnesium and calcium urinary excretion in the rat

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Modulation by calcitonin of magnesium and calcium urinary excretion in the rat. We evaluated the effects of human calcitonin (hCT) on electrolyte excretion in hormone-deprived rats, that is, in the absence of endogenous parathyroid hormone, antidiuretic hormone, thyrocalcitonin and glucagon, the effects of which might have interfered with those of exogenous calcitonin. Plasma hCT levels, measured by radioimmunoassay, varied from 0 to 32 ng/ml. In these rats, hCT decreased magnesium (Mg) and calcium (Ca) excretion in a dose-dependent fashion. Maximal decreases were observed for hCT plasma concentrations comprised between 3 and 5 ng/ml, and persisted at the highest doses. Sodium, potassium, water, and total solute excretions were constant in the calcitonin concentration range explored. The same was observed for phosphate, except that slight but significant phosphaturia was elicited by the highest doses. Calcium and phosphate infusions to attenuate the fall in plasma Ca and phosphate concentration subsequent to hCT infusion, did not alter the hormonal effect on Ca and Mg excretion. hCT can therefore directly modulate Mg and Ca reabsorption by the kidney at plasma concentrations within the physiological range. The maximal effects on Mg and Ca reabsorption were obtained at plasma concentrations which are generally reached after maximal stimulation of endogenous calcitonin secretion. It is suggested that in rats, endogenous secretion of calcitonin stimulates Ca and Mg renal reabsorption without modification of sodium and phosphate excretion.

Modulation de l'excrétion urinaire de magnesium et calcium par la calcitonine chez le rat. Les effets de la calcitonine humaine sur l'excrétion des électrolytes ont été étudiés chez le rat anhormonal, c'est-à-dire, en l'absence des hormones endogènes (hormone parathyroïdienne, hormone antidiurétique, calcitonine et glucagon) dont les effets auraient pu interférer avec ceux de la calcitonine exogène. La concentration plasmatique de calcitonine, mesurée par radioimmunologie, était comprise entre 0 et 32 ng/ml. Chez ces rats, l'excrétion du magnesium (Mg) et celle du calcium (Ca) était fonction de la concentration de calcitonine circulante. Ces excrétions atteignient leur valeur minimale pour des concentrations plasmatiques de calcitonin comprises entre 3 et 5 ng/ml, et se maintenaient à ce niveau aux concentrations plus élevées. L'excrétion du sodium, du potassium, du phosphate, de l'eau et des solutés totaux était constante et indépendante de la concentration de calcitonine circulante. Cependant aux concentrations plasmatiques les plus élevées, une phosphaturie modérée mais significative est apparue. La calcitonine peut donc moduler la réabsorption rénale de Mg et Ca quand son taux circulant varie dans les limites physiologiques. Les effets maximum sur la réabsorption de Mg et Ca furent obtenus pour des concentrations plasmatiques généralement atteintes après stimulation maximale de la sécrétion de calcitonine endogène. Il est suggéré que la sécrétion de calcitonine endogène induit chez le rat une augmentation de la réabsorption rénale du magnésium et du calcium sans modification de l'excrétion du sodium et du phosphate.

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The renal effects of calcitonin in mammals have been widely studied on both intact and thyroparathyroidectomized animals. It is now established that salmon, porcine, and human calcitonin (hCT) reduce the renal excretion of calcium (Ca) and magnesium (Mg). For human and salmon calcitonin, these effects result from stimulation of the transport of these two ions out of the thick ascending limb [1–3]. The thick ascending limb of Henle's loop is, however, a target site for many other hormones, including peptidic hormones such as vasopressin (ADH), glucagon, and parathyroid hormone (PTH) [4]. The renal effects of each of these hormones in the absence of a possible interference from the others [4] have recently been investigated in the rat. It was demonstrated on these hormone-deprived rats (DI Brattleboro rats lacking ADH, acutely thyroparathyroidectomized to suppress thyrocalcitonin and parathyroid hormone and given glucose [5] or somatostatin [3, 5, 6] to inhibit endogenous glucagon secretion) that each of the four hormones in question clearly enhanced renal reabsorption of Ca and Mg, and that these effects directly resulted from the stimulation of Ca and Mg transport both by the thick ascending limb [3, 5, 6] and, to a lesser extent, by the convolutions of the distal tubule accessible to micropuncture. Indeed, very recent micropuncture experiments clearly showed that dDAVP (a synthetic ADH analog), PTH, hCT, and glucagon enhanced Ca reabsorption and that PTH, hCT, and glucagon enhanced Mg reabsorption in this part of the distal tubule [7–9].

The purpose of this study was to establish dose-response relationships for the renal effects of calcitonin to determine the range of plasma concentrations within which variations in Ca and Mg excretion are observed. Administration of calcitonin lead to reductions of Ca and phosphate plasma concentrations. To prevent these considerable decreases the rats were given Ca and/or phosphate simultaneously with calcitonin infusions. In two groups of rats, however, the falls in plasma Ca and phosphate concentrations were not corrected so that the specific renal effects of calcitonin could be better distinguished from those resulting from modifications of the plasma composi-

tion. Human calcitonin was chosen because its chemical structure is very close to that of rat calcitonin [10] and because its immuno-reactivity is identical to that of rat calcitonin permitting precise plasma concentration determinations. The experiments were conducted on hormone-deprived rats to obtain the specific renal response of calcitonin in the absence of undesirable interference from the other peptidic hormones.

Methods

Clearance experiments were performed on 45 male rats with hereditary hypothalamic diabetes insipidus (DI), weighing 180 to 230 g and bred in our laboratory. Laboratory chow of constant composition (UAR France, Mg, 0.112 g; Ca, 0.97 g; P, 0.85 g per 100 g) was available to all rats until 17 hr before the experiments, at which time, the animals were put into metabolic cages to verify from their urine osmolality and output that they were homozygous (that is, DI). Free access to distilled water was allowed until anesthesia. Rats were anesthetized by the administration of 10 mg/100 g body wt of sodium 5 ethyl-5 (1' methyl-propyl)-2-thiobarbiturate (Inactin-Byk, Byk Gulden Konstanz, Federal Republic of Germany) and were then placed on a heated table to maintain body temperature at 37°C. Rats underwent tracheotomy, after thyroparathyroid glands were removed if necessary. For perfusion of solutions, one PE 50 catheter was inserted into the right jugular vein and another into the right femoral vein (and sometimes also into the left femoral vein). One PE 50 catheter was inserted into the right femoral artery for arterial blood sampling and PE 50 catheters were inserted into the left and right ureters for urine collection.

Estimated fluid losses were replaced by intravenous injections of 0.9% NaCl (1.5 ml/100 g body wt) administered once during the middle and once at the end of surgery. Then, at time zero, each animal was infused through the jugular catheter, at 10 $\mu l \cdot min^{-1}$/100 g body wt, with an 0.4% NaCl solution containing ^{3}H-inulin (infused at 0.2 μCi/min) somatostatin (Clin-Midy, 93537) and when necessary, calcitonin plus bovine serum albumin (1 mg/ml, BSA fraction V).

All animals were also infused through the right femoral vein catheter with a 0.4% NaCl solution at 30 μl/min/100 g, a rate fixed at the beginning of the experiments. The clearance periods began after a 60-min equilibration period during which urine samples from both kidneys were collected. Eight rat groups were studied. They comprised one group of intact DI rats, one of hormone-deprived rats and six of hormone-deprived rats infused with human calcitonin. The hormone-deprived rats were DI Brattleboro rats (that is, without circulating ADH) acutely thyroparathyroidectomized to suppress circulating PTH and calcitonin, and infused with somatostatin (70 ng/min/100 g body wt) to inhibit glucagon secretion [11]. Synthetic human calcitonin (Cibacalcin R, C47175 Ba) was administered to such hormone-deprived rats at rates of 0.10, 0.25, 1.00, and 2.5 mU/min/100 g body wt, respectively. The group receiving 2.5 mU/min/100 g body wt was given phosphate (50 mM Na_2HPO_4 and 10 mM Na H_2PO_4 at 5 μl/min/100 g body wt) through the jugular vein and calcium (50 mM $CaCl_2$, at 5 μl/min/100 g body wt) through the left femoral vein catheters. The group receiving 1.00 mU calcitonin/min/100 g body wt was given the same phosphate infusion, but the administration rate of calcium was halved (25 mM $CaCl_2$ and 31 mM NaCl at 5 μl/min/100 g body wt). These infusions were designed to prevent the plasma phosphate and calcium concentrations from falling after calcitonin administration. The reduction of Ca and Mg excretion subsequent to hCT administration was already maximal at 1.00 mU/min/100 g body wt (see **Results**). It was therefore of interest to explore the effects of hCT on the renal function at infusion rates of the hormone below 1.00 mU/min/100 g body wt, that is, at submaximal doses. To distinguish better between the direct hormonal effects on the tubular function and the indirect influence of the accompanying hypocalcemia and hypophosphatemia on these functions, four groups of hormone-deprived rats were studied. In two groups hCT was administered at 0.1 and 0.25 mU/min/100 g body wt without simultaneous Ca and phosphate infusions. At 0.1 mU, a large decrease in plasma Ca was observed, and a further decrease in plasma phosphate was noted at 0.25 mU. We decided to correct these modifications independently. In two other groups hCT was therefore administered at 0.1 and 0.25 mU/min/100 g body wt; at 0.1 mU the rats received a (25 mM $CaCl_2$, 31 mM NaCl) solution at 5 μl/min/100 g body wt to attenuate the plasma Ca decrease and at 0.25 mU the rats received a phosphate solution (50 mM Na_2HPO_4 + 10 mM Na H_2PO_4) at 5 μl/min/100 g body wt to correct the phosphate plasma decrease.

The experiments consisted of five 30-min clearance periods, the first of which started 210 min after tracheotomy. After each blood collection, samples were centrifuged, the plasma was separated and the red blood cells were resuspended for reinjection through the femoral vein catheter [5]. The urinary flow rate of both kidneys was determined after each urine collection and the rate at which the 0.4% NaCl solution was administered was adjusted to compensate for water losses. At the end of each experiment the hematocrit was measured and a blood sample taken for calcitonin radioimmunoassay. The kidneys were then removed and weighed after removal of their perirenal fat.

Analytical procedures

Plasma calcitonin was measured by an RIA described previously [12]. Briefly, synthetic human calcitonin (Ciba-Geigy AG Basel, Switzerland) was used for labeling with ^{125}I by the chloramide T method [13]. The labeled hormone was purified by gel filtration on Sephadex G-50 (fine). Specific activities of 250 to 350 μCi/μg were obtained routinely. The antiserum produced in a sheep against synthetic human calcitonin was shown to cross-react with rat calcitonin [12]. Barbital buffer (0.025 M; pH, 8.6) containing peptidase inhibitors was used as diluent. Incubation mixtures were prepared in duplicate in a total volume of 0.4 ml containing 0.02 ng labeled human calcitonin and a final dilution of the antiserum of 1:80,000 to give 40 to 60% binding of the labeled hormone; 0.1 ml of standard human calcitonin or of a sample with an unknown calcitonin concentration was added. Nonspecific binding was estimated in all assays for both unknown samples and standard curves (in the latter case, 0.1 ml of calcitonin-free plasma from thyroparathyroidectomized rats was added). Tubes were incubated in an equilibrium system at 4°C for 6 days. Bound and free radioactivity were separated on plasma-coated charcoal. The limit of detection of the assay was 0.15 ng calcitonin/ml plasma.

To determine the ^{3}H-inulin concentration 5-μl samples of urine and plasma were separately dissolved in Picofluor scintillating solution (Packard Instruments, Downers Grove, Illinois, USA), and radioactivity was measured by liquid scintillation

Table 1. Plasma concentration of calcitonin in hormone-deprived and hormone-deprived plus human calcitonin rats[a]

	Plasma calcitonin *ng/ml*
HD rats (8)	NS
HD + hCT *0.1 mU/min/100 g body wt* (11)	0.83 ± 0.09
HD + hCT *0.25 mU/min/100 g body wt* (11)	2.87 ± 0.21
HD + hCT *1.00 mU/min/100 g body wt* (5)	11.76 ± 2.13
HD + hCT *2.50 mU/min/100 g body wt* (3)	30.33 ± 1.20

Abbreviations: NS, not significantly different from the detection threshold of the assay (0.15 ng/ml); HD, hormone-deprived rats; hCT, human calcitonin.

[a] Values are mean ± SEM; the number of rats is in parentheses.

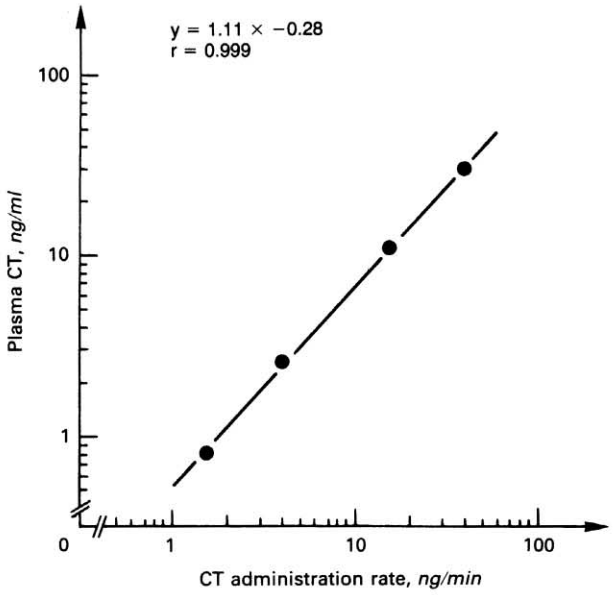

Fig. 1. *Correlation between immunoreactive plasma calcitonin and the administration rate of human calcitonin to hormone-deprived rats.* The *points* are mean values obtained from the 30 animals (see Table 1). SEM were smaller than the *point* diameters.

counting (Intertechnique SL 4000). Na and K concentrations were measured in both plasma and urine by flame-emission photometry (Netheler and Hinz), the Ca and Mg concentrations by atomic absorption photometry (Instrumentation Laboratory 353), and the phosphate concentration, by the method of Chen, Toribara, and Warner [14]. Osmotic pressure was determined in urine samples with a 5100 C vapor pressure osmometer (Wascor Inc.) and in plasma samples by microcryometry [15].

At the end of each experiment the water balance was estimated by comparing the amounts of water administered with the urinary losses; the difference was expressed as a percentage of the body weight. The values for all the parameters measured in plasma and urine were averaged and considered as a single datum for each animal. Data are presented as means ± SE. Results were analyzed by Student's unpaired *t* test.

Results

Table 1 gives the mean plasma calcitonin concentrations measured in five rat groups comprising hormone-deprived rats and hormone-deprived + hCT rats. As expected, the lowest concentration was observed in the hormone-deprived rats, and was not significantly different from the limit of detection of the radioimmunoassay (0.15 ng/ml). The concentration of circulating calcitonin rose with the administration rate of this hormone. The increase in the plasma calcitonin concentration was proportional to the dose (Fig. 1).

Tables 2, 3, and 4 present the data obtained in intact DI and hormone-deprived rats plus the two groups of hormone-deprived rats receiving the highest doses of hCT (1 and 2.5 mU/min/100 g body wt).

The water balance was not significantly different from zero in any of the four groups, indicating that the urinary water losses were adequately replaced (Table 2). This was corroborated by the unchanged hematocrit values which remained identical to

Table 2. Urinary flow rate, GFR, and water balance in intact diabetes insipidus rats, hormone-deprived rats and hormone-deprived rats receiving the two highest doses of human calcitonin[a]

	V *µl/min*	GFR/g kidney wt *µl/min*	Water balance *% body wt*
DI intact (5)	46.7 ±3.0	1023 ±107	0.6 ±0.5
HD rats (8)	41.1 ±8.0	850 ±46	0.9 ±0.9
HD + hCT (with Ca + Pi) *1.00 mU/min/100 g body wt* (5)	40.6 ±4.2	1230[c] ±41	0.9 ±0.6
HD + hCT (with Ca + Pi) *2.50 mU/min/100 g body wt* (5)	46.4 ±5.6	1115[b] ±81	0.6 ±0.3

Abbreviations: GFR/g kidney wt, glomerular filtration rate per gram of kidney weight; DI, diabetes insipidus; hCT, human calcitonin; Ca, calcium; Pi, inorganic phosphorus.

[a] Values from five clearance periods were averaged and considered as one datum for one animal. Means are given with their SE; the number of rats is in parentheses.

Unpaired *t* test between either hormone-deprived and intact, or hormone-deprived and hormone-deprived + hCT rats: [b] $0.025 < P < 0.05$; [c] $0.01 < P < 0.025$.

that of unanesthetized DI rats (0.45 ± 0.01, data not shown). As shown in Table 2, the glomerular filtration rate (GFR) was slightly but not significantly depressed by the hormone deprivation but was enhanced by administration of hCT. The urinary flow rate was high and was not changed by calcitonin, and fractional water excretion was roughly the same in all four groups. In the plasma (Table 3) the phosphate concentration was higher and the Mg and Ca concentrations were lower in

Table 3. Plasma composition (mmoles · liter^{-1} or mOsm · kg^{-1}) in intact diabetes insipidus rats, hormone-deprived rats and hormone-deprived rats receiving the two highest doses of human calcitonin[a]

	Na	Pi	Mg	Ca	K	Osm
DI intact	144	2.52[c]	0.72[c]	2.31[b]	4.00	311
(5)	±3	±0.22	±0.02	±0.03	±0.15	±2
HD rats	149	3.60	0.54	2.07	3.88	312
(8)	±3	±0.8	±0.02	±0.06	±0.22	±3
HD + hCT (with Ca+ Pi)	149	3.01[c]	0.63[b]	1.91	3.93	311
1.00 mU/min/100 g body wt	±3	±0.07	±0.02	±0.12	±0.14	±6
(5)						
HD ± hCT (with Ca + Pi)	149	3.19[c]	0.64	2.26	3.76	316
2.50 mU/min/100 g body wt	±3	±0.07	±0.05	±0.10	±0.13	±6
(5)						

Abbreviations: DI, diabetes insipidus; HD, hormone deprived; hCT, human calcitonin; Ca, calcium; Pi, inorganic phosphorus; Na, sodium; Mg, magnesium; K, potassium; Osm, osmolality.

[a] Values from five clearance periods were averaged and considered as one datum for one animal. Means are given with their SE; the number of rats is in parentheses.

Unpaired *t* test between either hormone-deprived and intact, or hormone-deprived and hormone-deprived + human calcitonin rats: [b] $P < 0.05$; [c] $P < 0.01$.

Table 4. Electrolyte and total solute excretion rate (nmoles · min^{-1} or mOsm · min^{-1}) in intact diabetes insipidus rats, hormone-deprived rats and hormone-deprived rats receiving the two highest doses of human calcitonin[a]

	Na	Pi	Mg	Ca	K	Osm
DI intact	783	398[b]	43.1	5.7[b]	532	5301
(5)	±113	±37	±1.5	±1.8	±149	±222
HD rats	1425	42	44.1	55.0	527	7059
(8)	±749	±19	±6.0	±10.3	±84	±1358
HD + hCT (with Ca+ Pi)	827	76	8.8[b]	5.3[b]	405	6376
1.00 mU/min/100 g body wt	±327	±5	±2.0	±1.3	±56	±468
(5)						
HD ± hCT (with Ca + Pi)	1931	182[b]	10.2[b]	6.4[b]	573	8403
2.50 mU/min/100 g body wt	±403	±46	±2.5	±3.1	±96	±1362
(5)						

Abbreviations are the same as those used in Table 3.

[a] Values from five clearance periods were averaged and considered as one datum for one animal. Means are given with their SE.

Unpaired *t* test between either hormone-deprived and intact or hormone-deprived and hormone-deprived plus human calcitonin rats: [b] $P < 0.01$.

hormone-deprived than in intact DI animals, as already observed [5]. Between the hormone-deprived and hormone-deprived + hCT rats, no significant differences were found as regards Na, K, and total solutes. The administration of hCT to such hormone-deprived rats tended to raise the plasma Mg concentration but to values lower than those of intact DI rats. The plasma Ca concentration did not differ significantly from that of the hormone-deprived group; the phosphate concentrations were lower than those of this latter group, but phosphatemia was nevertheless high (higher than that of intact DI rats).

Table 5. Urinary flow rate, GFR, and water balance in hormone-deprived rats receiving the two lowest doses of human calcitonin

	V *μl/min*	GFR/g kidney wt *μl/min*	Water balance *% body wt*
HD + hCT (without Ca)	50.1	1019	0.4
0.1 mU/min/100 g body wt	±5.7	±41	±0.5
(7)	NS	NS	NS
HD + hCT (with Ca)	51.9	1002	0.2
1.00 mU/min/100 g body wt	±4.5	±49	±0.5
(4)			
HD + hCT (without Pi)	39.0	1107	−0.2
0.25 mU/min/100 g body wt	±6.2	±57	±0.7
(5)	NS	NS	NS
HD + hCT (with Pi)	39.2	1045	1.8
0.25 mU/min/100 g body wt	±5.0	±42	±0.9
(6)			

Abbreviations: NS, not significant; other abbreviations are the same as those used in Tables 2 and 3.

[a] Values from five clearance periods were averaged and considered as one datum for one animal. Means are given with their SE; the number of rats is in parentheses. Unpaired *t* test between HD + hCT with versus without Ca and with versus without P.

Hormone deprivation and administration of calcitonin considerably modified the excretion of electrolytes (Table 4). For magnesium, the excretion in the two groups receiving the largest doses of calcitonin was significantly lower than in the hormone-deprived group. Hormone-deprivation consistently raised calcium excretion, and administration of calcitonin reduced it to the rate measured in the intact DI rats. As regards Na, K, and total solutes, excretion rates did not differ significantly from one group to another. Phosphate excretion fell considerably in the hormone-deprived animals. hCT at 1.0 mU/min/100 g body wt did not modify this excretion. At 2.5 mU/min/100 g body wt a phosphaturic effect was noted.

Tables 5 and 6 present the data obtained from hormone-deprived rats receiving the lowest doses of hCT (0.1 and 0.25 mU/min/100 g body wt) infused or not with Ca or phosphate. Table 5 shows that the urinary flow rate and the glomerular filtration rates were very similar, irrespective of the administration of Ca or phosphate. The water balance was close to zero in all four groups. Table 6 indicates the plasma composition and the electrolyte excretion rates. In all four groups the magnesemia was similar. The Mg and Ca excretion rates were not significantly different whether the drop in calcemia (0.1 mU groups) or in phosphatemia (0.25 mU groups) was corrected or not. In addition, these excretion rates were significantly lower in the 0.1 mU groups than in the hormone-deprived animals and even lower in the 0.25 mU groups. Phosphaturia was as low as that observed in the hormone-deprived rats, except in the 0.25 mU group which received a phosphate infusion. In this group, as in the 1.0 and 2.5 mU groups that also received phosphate, the phosphaturia was extremely variable from one animal to another. The plasma concentration and excretion rates of Na, K, and total solutes were similar to those observed in the hormone-deprived rats (data not shown).

In conclusion, it is clear that the reductions in Ca and Mg

Table 6. Plasma composition (P, mmoles/liter) and electrolyte excretion rate (UV, nmoles/min) in hormone-deprived rats receiving the two lowest doses of human calcitonin[a]

	HD + hCT *0.1 mU/min/100 body wt*			HC + hCT *0.25 mU/min/100 g body wt*		
	Without Ca (7)	*P*	With Ca (4)	Without Pi (5)	*P*	With Pi (6)
P_{Pi}	3.08 ±0.11	NS	3.08 ±0.05	2.79 ±0.10	<0.01	3.48 ±0.13
UV_{Pi}	11.9 ±9.4	NS	4.1 ±3.0	10.0 ±7.6	NS	67.7 ±37.5
P_{Mg}	0.57 ±0.02	NS	0.62 ±0.04	0.61 ±0.04	NS	0.56 ±0.03
UV_{Mg}	33.7 ±2.1	NS	25.5 ±6.0	10.4 ±5.2	NS	11.0 ±4.5
P_{Ca}	1.62 ±0.03	<0.001	1.97 ±0.06	1.78 ±0.06	NS	1.62 ±0.05
UV_{Ca}	19.5 ±4.5	NS	27.6 ±7.4	5.6 ±2.9	NS	9.5 ±3.3

[a] Values from five clearance periods were averaged and considered as one datum for one animal. Means are given with their SE. Unpaired *t* test between HD + hCT with versus without Ca and with versus without Pi. The number of rats is in parentheses. See Table 3 for abbreviations.

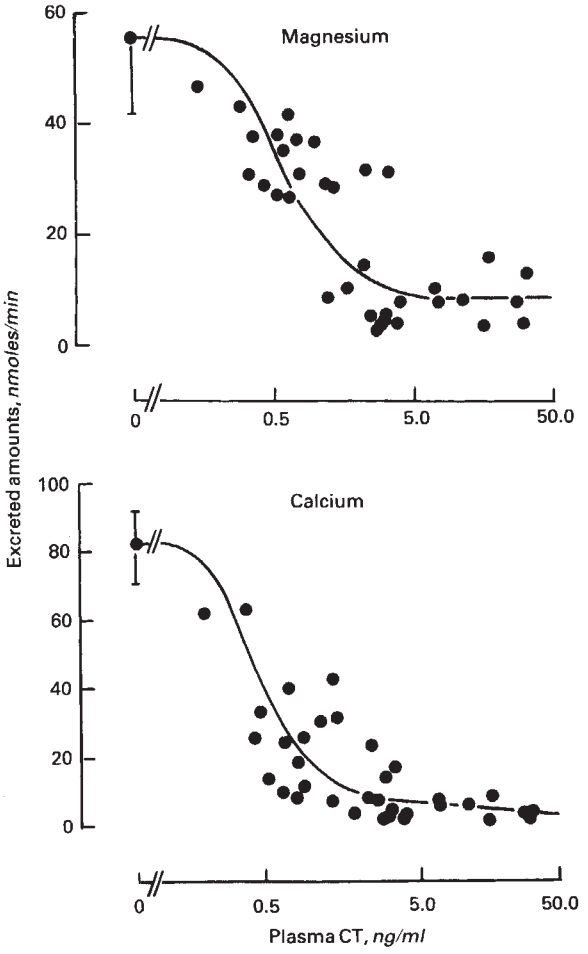

Fig. 2. *Relationship between Mg and Ca excretion by the kidney and plasma immunoreactive calcitonin.* Each *point* is the mean value of five clearance periods from different animals. Mean values for rats with a plasma CT less than 0.15 ng/ml are given with their SE.

excretion are the results of a direct effect of CT on the tubular functions. As illustrated in Figure 2, the renal excretion of Mg and Ca was clearly related to the plasma CT concentration. The excretion rates followed a dose response curve. Between 0 and 5 ng/ml, Mg and Ca excretion decreased sharply and then leveled off. This indicates that Ca and Mg tubular reabsorptions were maximally stimulated when the plasma hCT concentration was around 5 ng/ml.

Discussion

The present study demonstrates that synthetic human calcitonin produced maximal reductions in calcium and magnesium excretion in hormone-deprived rats when plasma calcitonin concentrations reached 3 to 5 ng/ml. Above these levels and up to 30 ng/ml, no further changes in Ca and Mg excretion were observed. Na, K, or total solute excretion remained roughly constant, irrespective of the plasma calcitonin concentration. Phosphate excretion was low in these hormone-deprived animals because of parathyroid gland ablation, but tended to rise when the plasma calcitonin exceeded 15 ng/ml.

Calcitonin increased the GFR, as has been frequently observed [1, 3, 16, 17]. This increase was accompanied by a rise in the filtered load of electrolytes. Nevertheless, Ca and Mg excretion gradually fell as a function of the circulating level of calcitonin. Calcitonin administration generally induces marked hypocalcemia. It appears that hypocalcemia could enhance sodium chloride reabsorption in the thick ascending limb [2]. Two mechanisms could be involved. There is evidence that cytostolic Ca activity may directly alter the rate of luminal sodium entry [18], and recently Di Stefano et al [19] demonstrated that an increase of the extracellular Ca concentration inhibits active sodium chloride reabsorption in microperfused rabbit cortical thick ascending limb by a reduction in paracellular Na^+ conductance. It is conceivable, therefore, that hypocalcemia is accompanied by a rise of sodium chloride transport and hence of Ca and Mg reabsorption if, as is likely, sodium chloride and divalent cation transports by the thick ascending limb are linked. Such a rise could be responsible for the reduction of Mg and Ca urinary output during hCT administration. The present experiments, however, show that the decreases in Ca and phosphate plasma concentration were not sufficient to significantly influence the Ca and Mg excretion rates. This was already observed by Carney and Thomson [17] who reported that calcium infusion prior to salmon calcitonin administration to prevent a detectable drop in plasma Ca concentration did not influence the reduction of Ca and Mg excretion. It is therefore safe to conclude that the reduction in Mg and Ca excretion was the result of a direct effect of calcitonin on the kidney. This conclusion is supported by the existence along the nephron of target sites for calcitonin [20], including, in the rat, the thick ascending limb, the distal convoluted tubule, and the cortical collecting ducts [21]. It is further corroborated by recent data which demonstrate that in

hormone-deprived rats, calcitonin, like ADH, stimulates calcium and magnesium reabsorption mainly in the loop of Henle [3] and, to a lesser extent in the part of the distal tubule accessible to micropuncture [7].

Mg and Ca reabsorption by the kidney was maximally stimulated, as already mentioned, when the plasma calcitonin concentration reached 3 to 5 ng/ml. In the normal rat, full stimulation of endogenous calcitonin release by calcium infusion increased the level of circulating calcitonin to values around 10 ng/ml [22]. The fact that variations of the plasma calcitonin concentration within physiological limits were observed to modulate Mg and Ca transport by the kidney is satisfactory. In DI nonfasted Brattleboro rats, the basal calcitonin concentration is 0.62 ± 0.09 ng/ml ($N = 5$) (unpublished observations), a value which confirms previous measurements by others in this strain [23]. This probably explains in part why Ca excretion was lower in intact DI rats than in the hormone-deprived rats. The influence of the other peptidic hormones, that is, PTH and glucagon, known to stimulate Ca and Mg reabsorption in the loop of Henle [6] and the distal tubule [9], cannot be excluded.

It is now clearly established that moderate doses of salmon and mammalian calcitonins can reduce renal Mg and Ca excretion [3, 17, 24, 25]. The effects of calcitonins on Na and phosphate excretion were very often controversial. Some authors found that calcitonin did not affect excretion of these two ions, whereas others observed either phosphaturia or natriuresis, or both (see [17]). In some instances, calcitonin even reduced phosphaturia [1]. As is now generally acknowledged, these conflicting results could be due to the doses [17, 24] and/or the nature of the hormonal preparation used. It is possible that the discrepancies were also due to the interference of the other peptidic hormones which act via activation of the adenylate-cyclase system.

Williams et al [24] examined, in intact rats, the dose-response relationship of human calcitonin on the renal handling of electrolytes. The administration rate ranged from 0.4 to 24 mU/min/100 g body wt. In these rats, the decrease in Mg fractional excretion was already maximal at 0.4 mU/min/100 g body wt, which agrees with our data, whereas calcium excretion only declined after doses equal to or higher than 1.6 mU/min/100 g body wt. Phosphate and sodium excretion were unaltered, irrespective of the calcitonin dose.

In other investigations of such dose-response relationships, salmon or porcine calcitonin was used [17, 24–26]. According to Williams et al [24], the action of salmon calcitonin on renal electrolyte excretion in the rat compared with that of human calcitonin might be different from the relative hypocalcemic potencies of the two hormones. In addition, we found that salmon calcitonin administered at 2.5 mU/min/100 g body wt to hormone-deprived rats reduced both the absolute and the fractional excretion of water, despite a concomitant increase in GFR, and raised the urinary osmolality [27]. Such an antidiuretic response to salmon calcitonin administration was also reported by Carney et al [28]. As indicated here, human calcitonin administered at the same rate as salmon calcitonin was without effect on both water excretion and urine osmolality. The precise renal activity of salmon calcitonin in relation to that of human calcitonin, therefore, remains to be established. Almost nothing is known about the relative potencies of human and porcine calcitonin. It is therefore difficult to draw precise conclusions from a comparison of our data with those obtained for salmon or porcine calcitonin [17, 25, 26].

Finally, this work shows that in hormone-deprived rats, variations in the plasma calcitonin concentration within the physiological range can modulate Ca and Mg excretion. It is very likely that such a precise dose-response relationship was obtained thanks to the absence of the other peptidic hormones (ADH, PTH, and glucagon) which can also stimulate Mg and Ca reabsorption in the thick ascending limb and the distal tubule. In the intact animal, the presence of these hormones would certainly have obscured the response, as illustrated by the fact that Ca excretion was already at its minimum in the intact DI rats. Administration of exogenous calcitonin could not feasibly reduce Ca excretion further. In intact antidiuretic Wistar rats, we previously observed that fractional Mg excretion was very low [1]. There again, the possibility that exogenous calcitonin could further reduce Mg excretion is very slight. Hence, one might question the physiological significance of our observations. That the effects of calcitonin ([3] and present study), ADH [5], and glucagon [6] on renal thick ascending limb and distal tubule [7–9] functions were shown at plasma concentrations within the physiological range provides a necessary condition for such effects to be of significance. In addition, it must be emphasized that, although the stimulatory effect of ADH on Mg reabsorption by the kidney was first revealed in hormone-deprived animals identical to those used in the present study [5], it has since been clearly demonstrated that ADH either when administered acutely [29] or chronically [30] acts also on Mg excretion in intact DI Brattleboro rats. By analogy we therefore believe that the sharp modulation by calcitonin of renal Ca and Mg excretion observed in hormone-deprived rats is not devoid of physiological significance, and a search for the physiological or physiopathological conditions under which this modulation might be expressed is clearly warranted.

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