Urinary Excretion of Cyclic Nucleotides and Phosphate in Response to Parathyroid Hormone and Calcitonin in Man

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Summary

The response to parathyroid hormone (PTH) and calcitonin (CT) was studied in eight children with various bone diseases by determining the serum calcium (Ca) and phosphate (P) concentration, urinary phosphate excretion rate, renal phosphate clearance, the percentage of filtered phosphate reabsorbed by the renal tubule (%TRP), creatinine clearance (Ccr), urinary cyclic adenosine 3',5' monophosphate excretion rate (U_{cAMPV}), and urinary cyclic guanosine 3',5' monophosphate excretion rate (U_{cGMPV}). Administration of PTH caused no significant change in serum Ca and P values, whereas CT produced a decrease in Ca (Δ Ca, -1.4 ± 0.1 mg/100 ml) and P (Δ P; -1.1 ± 0.1 mg/100 ml). There was an increase in U_{cAMPV} (ΔU_{cAMPV} ; 437 ± 74 nmoles/min/100 ml Ccr) without any significant change in U_{cGMPV} after administration of PTH. Phosphaturia was produced by both PTH (Δ TRP, $-18 \pm 3\%$) and CT (Δ TRP, $-13 \pm 2\%$). However, CT did not elicit any increase in either U_{cAMPV} or U_{cGMPV} .

Speculation

Parathyroid hormone (PTH) and calcitonin (CT) induce phosphaturia of a similar magnitude whereas PTH alone increased the urinary excretion of cyclic adenosine 3',5' monophosphate (cAMP). The phosphaturia induced by CT may not be mediated by a similar mechanism involving the adenylate cyclase-cAMP system as demonstrated for PTH.

Parathyroid hormone (PTH) and calcitonin (CT) have opposing effects on bone resorption; PTH induces hypercalcemia by stimulating bone resorption whereas CT induces hypocalcemia by inhibiting bone resorption. Despite their opposing effects on bone, they both act on the kidney to produce phosphaturia. The actions of PTH and CT on both bone and kidney are presumably mediated through the adenylate cyclase cyclic adenosine 3',5'-monophosphate (cAMP) system. Studies have shown increased renal formation and urinary excretion of cAMP in response to PTH (11, 12). This change in the urinary excretion of cAMP is considered a qualitative indicator of the effect of PTH on the kidney (8, 22, 32). Some studies also have shown an increase in the formation of cAMP in the kidney after CT administration (20, 27). However, few studies have investigated urinary excretion of cAMP after CT administration, and those that are available, report conflicting results. No increase in urinary excretion of cAMP was reported after CT administration in parathyroidectomized rats, dogs (11), and in one patient with hypoparathyroidism (28), whereas an increase in cAMP was reported in hamsters (23) and parathyroidectomized rats (24). Some studies in man (1, 2, 28) have shown an increase in urinary cAMP in response to salmon or porcine CT, but the magnitude of rise was insignificant when compared to the

rise after PTH. Therefore, to assess the relationship of urinary cAMP excretion and the phosphaturic response to CT in man, we have studied the effect of synthetic human CT on patients with various bone diseases, all of whom had normal phosphaturic and cAMPuric responses to PTH. In view of the possible role of cyclic guanosine 3',5'-monophosphate (cGMP), as demonstrated by its potent effect on *in vitro* renal phosphate uptake (31) and increased urinary excretion of cGMP in response to PTH in man (28) and in response to CT in rats (17), we also assessed the urinary excretion of cGMP in response to PTH and CT in our patients.

MATERIALS AND METHODS

EXPERIMENTAL SUBJECTS

Eight children who were admitted to Shriners Hospitals, Honolulu Unit with various bone diseases were studied. Clinical data of these patients are summarized in Table 1. All had normal serum calcium (Ca) and phosphate (P) values at the time of the study and showed normal renal response to PTH.

METHODS

Assessment of Responses to PTH. Response to PTH was assessed by determining serum Ca and P concentration, renal P handling, and urinary excretion of cAMP and cGMP before and after IV injection of bovine parathyroid extract (PTE; Eli Lilly & Co., Indianapolis, IN). Food was withheld during each test commencing the previous evening. One hr before starting urine collection, each patient was given 20 ml of water per kg of body weight to induce diuresis. Subsequently, water intake was regulated to match urine output until the end of the test. Blood samples were obtained hourly throughout the test and analyzed for Ca, P, and creatinine concentrations. Urine was collected hourly for 3 hr, after which PTE was injected; thereafter, urine was collected half hourly for 1 hr and hourly for the remaining 3 hr of the test. Urine specimens were collected and frozen at -20°C immediately. These were subsequently analyzed for P, cAMP, cGMP, and creatinine. The dose of PTE given was 8 U.S.P. units/kg of body weight diluted in 30 ml of normal saline and injected IV over a 10-min period. Urinary P excretion rate (UpV), renal P clearance (C_p), the percentage of filtered P reabsorbed by the renal tubule (%TRP), creatinine clearance (Ccr), urinary cAMP excretion rate (U_{cAMPV}), and urinary cGMP excretion rate (U_{cGMPV}) were determined for each collection period. The values of UpV , U_{cAMPV} , U_{cGMPV} were corrected per 100 ml of Ccr to allow comparison between patients. Mean value of each serum sample obtained before PTE infusion in each patient was taken as pre-test value for that patient and the maximum value obtained during the period after PTE infusion was taken as the post-test value.

Table 1. Summary of clinical data

Patient	Sex	Age (yr)	Diagnosis	Serum Ca (mg %)	Serum P (mg %)	Serum alkaline phosphatase (mu/ml)	Medication
1	M	17	Congenital hyperphosphatasia	9.6	4.5	2760	None
2	F	18	Congenital hyperphosphatasia	9.5	4.7	1300	None
3	F	12	Vitamin D-dependent rickets	9.2	6.5	226	1,25(OH) ₂ D ₃ (1.0 µg/day)
4	M	12	Vitamin D-dependent rickets	9.2	5.6	440	1,25(OH) ₂ D ₃ (1.0 µg/day)
5	M	15	Vitamin D-dependent rickets	8.9	4.6	296	Vitamin D ₂ (50,000 u/day)
6	F	14	Bone cyst, right femur	9.9	4.2	90	None
7	M	12	Chronic osteomyelitis	9.4	5.5	300	Penicillin
8	M	6	Multiple enchondromatosis	9.4	5.3	124	None

Assessment of Responses to CT. Response to CT was assessed by the same protocol as for PTH except that synthetic human calcitonin (Ciba-Geigy Corp., Summit, NJ) was injected instead of PTE. The dose of CT given was 0.5 mg (35) diluted in 30 ml normal saline and injected IV over a 10-min period. The interval between PTE and CT tests was at least 1 wk for each patient.

Analytical Methods. Serum Ca was measured by an automated Ca analyzer using a fluorometric titration of EGTA as the Ca chelator (34). P in serum and urine was determined by the method described by Goldenberg and Fernandez (18). Creatinine in serum and urine was determined using a modified Jaffe reaction (33). The urinary concentration of cAMP was determined by a modified protein binding method as previously described (29). The urinary concentration of cGMP was determined using a radioimmunoassay kit purchased from New England Nuclear, Inc.

Statistical Analysis. The results for all patients were given as mean ± S.E. Results were analyzed for significance using Student's *t* test (14).

The protocol for this investigation was approved by the human experimentation committee, Department of Pediatrics, John A. Burns School of Medicine, University of Hawaii; an informed consent was obtained from the guardian or the patient.

RESULTS

Serum Ca and P responses to PTE and CT are shown in Table 2. Acute administration of PTE caused no significant changes in serum Ca and P, whereas the administration of CT caused a decrease in serum Ca, reaching a maximum in 3 to 4 hr, with an average drop of 1.4 mg % in all eight children. CT also produced a significant decrease in serum P value, reaching a maximum in 3 to 4 hr.

Figure 1 shows renal responses produced by PTE and CT. PTE and CT both produced a prompt and significant increase in urinary P excretion. PTE increased UpV, Cp, and decreased %TRP. These changes were maximal in the second half-hour after PTE infusion. PTE produced a prompt and marked increase in the urinary cAMP (ΔU_{cAMPV} , 437 ± 74 nmol/min/100 ml Ccr). This increase in urinary cAMP excretion always occurred in the first half hour after PTE administration. There was no significant increase in urinary excretion of cGMP after PTE. After CT, there was a significant increase in UpV, Cp, and decrease in %TRP. These changes were similar in magnitude to those after PTE. However, after CT administration, the duration of increased P excretion was prolonged as compared to that of PTE administration, and there was no increase in either cAMP or cGMP excretion.

DISCUSSION

PTE and CT tests were carried out while all patients were normocalcemic and normophosphatemic, and their pre-test renal handling of P was comparable to those observed in normal subjects (6). Some of our patients were receiving vitamin D or 1, 25 (OH)₂ vitamin D₃ therapy (Table 1, Nos. 3 to 5). Their serum PTH, 25

Table 2. Serum Ca and P responses to PTE and CT in eight patients

Hormone		Ca (mg %)	P (mg %)
PTE	Pre ¹	9.0 ± 0.2 ²	4.7 ± 0.3
	Post	9.6 ± 0.1	4.2 ± 0.5
CT	Pre	9.1 ± 0.2	4.6 ± 0.3
	Post	7.7 ± 0.3 ³	3.5 ± 0.2 ³

¹ See the text for an explanation of Pre and Post values.

² Mean ± S.E.

³ *P* < 0.05 when compared to Pre infusion value.

Fig. 1. Renal response to PTE and CT in eight patients. The effect of PTE (8 U.S.P. units/kg IV) (left panel) and CT (0.5 mg IV) (right panel) on UpV, Cp, %TRP, U_{cAMPV} , and U_{cGMPV} . Urine samples were collected hourly for 3 hr before the administration of PTE or CT and thereafter half hourly for 1 hr and hourly for the remaining 2 hr of the test. Notice the magnitude of changes in UpV, Cp, and %TRP after hormone administration is similar, but after CT, the effect is more prolonged, and there was no rise in U_{cAMPV} . Both PTE and CT induced no rise in U_{cGMPV} .

(OH) vitamin D, and 1, 25 (OH)₂ vitamin D levels were all within normal limits. When PTE was infused, there was a prompt increase in urinary cAMP excretion during the first 30 min. This increase was followed by phosphaturia as documented by UpV, Cp, and %TRP, and these changes were comparable to those observed in normal subjects (5, 12, 13). However, when they were infused with CT, there was no significant increase in urinary excretion of cAMP in spite of similar but prolonged changes in UpV, Cp, and %TRP. Several studies have documented the role of cAMP in mediating the phosphaturic response of PTH at the kidney level in man and experimental animals (11, 12, 16, 25, 30). Administration of cAMP has been shown to mimic the phosphaturic effect of PTH *in vivo* (4, 30), and *in vitro* (3, 15). An increase in adenylate cyclase activity and accumulation of intracellular cAMP after PTH administration have been demonstrated (16, 26). The intracellular accumulation of cAMP and subsequent leakage from the renal tubular cells is considered to be an explanation for the increase in urinary excretion of cAMP, and thus, the latter change has been used as an indicator of the renal responsiveness to PTH (3, 8, 22, 32). Our study confirms the earlier reports of a phosphaturic response to CT in both humans (1, 2, 7, 19) and rats (24). This phosphaturic effect of CT is assumed to be mediated by the stimulation of adenylate cyclase-cAMP system, but studies on the urinary excretion of cAMP in response to CT are not conclusive. Few investigators have studied the excretion of urinary cAMP after CT administration in man. In a study of the natriuretic effect of CT in man, Bijvoet *et al.* (7) demonstrated no urinary increase in cAMP after acute administration of CT in one patient with Paget's disease and another with osteoporosis. Despite the lack of cAMP response, these two patients demonstrated a phosphaturic response. Another study (28) demonstrated no increase in urinary cAMP excretion after CT in a patient with hypoparathyroidism, but an increase was noted in a patient with Paget's disease. This rise in cAMP was interpreted to be secondary to an increase in PTH secretion. Our study examined the phosphaturic effect and urinary excretion of cAMP after the administration of CT, comparing them with the results obtained after PTE in the same patients. We have shown that after CT administration, there was no significant increase in cAMP, in spite of a phosphaturic response which was comparable in magnitude to that of PTE.

Although we have not determined serum PTH levels after CT administration, the observed phosphaturic response to CT seems to be not due to a secondary rise in PTH level because there was no increase of urinary cAMP during the entire period of the test, and the maximum hypocalcemia occurred after 3 to 4 hr which was later than the phosphaturia that occurred in 1 to 2 hr after administration of CT. Adachi *et al.* (1) observed only a slight increase in urinary cAMP as well as plasma PTH in one patient treated with porcine CT. However, there was no correlation between the rise in PTH and urinary cAMP; urinary cAMP rise occurred immediately, and the PTH rise occurred 4 hr after CT infusion. Ardaillou *et al.* (2) have observed an increase in plasma levels of cAMP and urinary excretion of cAMP in response to salmon CT. Inasmuch as there was no change in plasma PTH level, it was concluded by them that the effect of salmon CT was direct and not a consequence of PTH secretion. It should be emphasized that an increase in excretion of urinary cAMP observed by these investigators (1, 2) and others (24, 28) in response to CT was minimum in magnitude as compared to the response elicited by PTH. In our study, we used synthetic human CT, which has a potency of 75 to 100 MRC units/mg. The possibility that the lack of increase in urinary cAMP excretion was due to a lower potency of synthetic human CT was considered, but this seems unlikely in view of the report of Hass *et al.* (19), who have shown a similar magnitude of renal responses elicited by CT of various species, including human.

In view of the possible role of cGMP (17) in mediating the renal responses of PTH and CT, we determined the urinary excretion of cGMP. Neither PTE nor CT altered the urinary excretion of cGMP.

The lack of increase in the urinary excretion of cAMP after CT may be explained on the basis of several mechanisms: (1) only a minute quantity of cAMP may be required to elicit a phosphaturic response, and CT could produce this effect without a renal leakage of cAMP; (2) although CT may increase intracellular production of cAMP, the latter may not be excreted due to a rapid intracellular degradation by phosphodiesterase; (3) inasmuch as receptors for CT in the nephron are reported to be located at sites different from those of PTH (9, 10, 12, 21), cAMP produced by CT may be trapped in compartments impermeable to this nucleotide; or (4) CT may act directly at the renal cells through a cAMP independent system to block P reabsorption. The latter view is exemplified by Ardaillou *et al.* (2), who have demonstrated that the source of increased cAMP in urine after CT administration was not from the tubular cells but from the plasma.

CONCLUSION

Administration of CT induces phosphaturia, similar in magnitude to that of PTH but unlike PTH this phosphaturia is not preceded by an increase in urinary excretion of cAMP. Our study indicated that renal excretion of cAMP following CT administration is different from that of PTH. It is possible that the phosphaturia induced by CT may not be mediated by a similar mechanism involving the adenylate cyclase-cAMP system as demonstrated for PTH. Neither PTH nor CT influence urinary excretion of cGMP.

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