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Vitamin D and Cyclic Nucleotide Changes in Response to Calcitonin in Man

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ABSTRACT

Vitamin D and cyclic AMP changes in response to calcitonin injection were studies in three normal adults and one patient with osteogenesis imperfecta. Plasma 1,25-$(OH)_2$D levels increased in one normal adult and the patient. Plasma 25-OHD levels did not change. Plasma cyclic AMP was decreased in all four subjects, but urine cyclic AMP did not change. These results suggest that calcitonin may affect plasma cyclic AMP and may have some affect on renal 1α-hydroxylation in humans.

There have been several studies on the effect of calcitonin (CT) on vitamin D and cyclic AMP (cAMP) metabolism. However, its exact role is still controversial[1-10,15]. Recently we observed that plasma 1,25-$(OH)_2$D levels were clearly depressed during the first month of CT therapy and then became and remained normal in patients with osteogenesis imperfecta (OI)[9]. Thus, the acute and chronic effects of CT on vitamin D metabolism in humans may differ. The present study was undertaken to evaluate the acute effect of CT on vitmain D metabolism and cAMP in healthy adults and one patient with OI.

MATERIALS AND METHODS

Three healthy male volunteers aged 31, 33 and 35 years and one patient with OI aged 14 years were studied after informed consent was obtained. They were instructed to fast from 9 p.m. of the previous day to 12 p.m. of the day of examination. At 9 a.m., blood was drawn and urine was collected, and 100 Medical Research Council units of porcine calcitonin (Armour Pharmaceutical Company) was injected intramuscularly. CT was injected 1.5 months after the cessation of CT therapy in one OI patient. Blood and 1-hr urine samples were collected before injection and 3, 6, 12 and 24 hr later. Blood samples were analyzed for 1,25-$(OH)_2$D, 25-OHD, cAMP, PTH, Ca, P, and creatinine. Urine samples were analyzed for cAMP, Ca, P, and creatinine. The diet contained adequate amounts of Ca, P, and vitamin D. Ca, P, and creatinine in serum and urine were measured by standard autoanalyzer techniques. The percent tubular reabsorption of phosphorus (%TRP) was calculated. Serum PTH was measured by RIA with an antibody (Immuno Nuclear Corporation, USA) recognizing the C-terminal PTH. Serum PTH levels in normal adults were less than 0.5 ng/ml. cAMP was determined by RIA (Yamasa Shouyu cAMP kit, Japan). CAMP levels in plasma and urine in normal adults were 18-40 pmol/ml and 4-15 μmol/g creatinine, respectively. Plasma 25-OHD and 1,25-$(OH)_2$D were measured by competitive protein-binding assay, as previously described by our group[11-14]. The adult control values during entire year were: mean normal value of 25-OHD, 20.4 ± 9.0 ng/ml [range, 8.4-38.6 ng/ml; n=20]; and mean normal value of 1,25-$(OH)_2$D, 36.8 ± 16.0 pg/ml [range, 12.0-69.7 pg/ml; n=25][11]. Samples for each

study were processed in the same assay.

RESULTS

The changes of the metabolites of vitamin D after CT injection are showing in Fig. 1. Plasma 1,25-$(OH)_2$D concentrations were increased in one normal adult and the OI patient. Plasma 25-OHD values remained normal after CT injection in all four subjects. Plasma cAMP values were decreased 6-12 hr after injection in all subjects (Fig. 2). Urinary excretion of cAMP did not change following CT administration in all subjects. Serum Ca levels were decreased 6-12 hr after injection in two controls and after 3 hr in the patient. Serum PTH values were increased in these three subjects. Serum levels of P did not change after injection. Urinary excretion of Ca were increased after 3 hr in two controls and after 12 hr in the patient. Those of P were slightly increased after 12 hr in all controls and after 3-12 hr in the patient with OI. %TRP was decreased 3-12 hr in all four subjects.

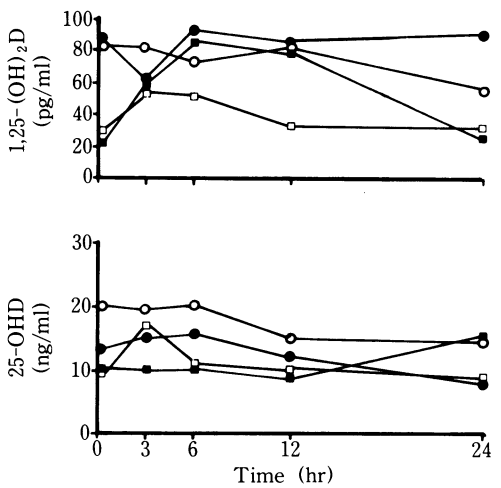

Fig. 1. Influence of CT on plasma 1,25-$(OH)_2$D and 25-OHD in three healthy adults (○, ●, □) and one patient with osteogenesis imperfecta (■).

DISCUSSION

The effect of CT on vitamin D metabolism is controversial, although several animal studies and three investigations in humans, including our previous study, have been reported[1-10,15]. In animal studies, Rasmussen et al[10] observed that

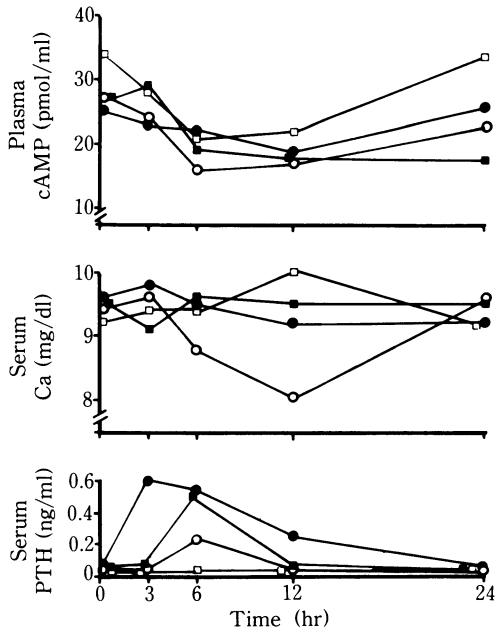

Fig. 2. Influence of CT on plasma cAMP and serum PTH and Ca in three healthy adults (○, ●, □) and one patient with osteogenesis imperfecta (■).

in vitro administration of CT depressed 1,25-$(OH)_2$D synthesis in isolated chick renal tubules. Kawashima et al[6] reported that *in vitro* CT selectively stimulated 1-hydroxylation in the proximal straight tubules of vitamin D-deficient rat kidney by a mechanism independent of adenylate cyclase activation.

In vivo studies of CT's effect on vitamin D metabolism have also been reported[3,8,10]. CT stimulated the *in vivo* conversion of 25-OHD into 1,25-$(OH)_2$D in vitamin D-deficient rats with an intact thyroparathyroid system, but this CT effect disappeared when the parathyroid glands were removed[8]. These results indicated that the stimulatory effect of CT on 1,25-$(OH)_2$D synthesis was due mainly to a secondary response to the parathyroid glands. However, Horiuchi et al[5] observed that CT enhanced *in vivo* 1,25-$(OH)_2$D synthesis even in vitamin D-deficient thyroparathyoidectomized rats and that this stimulatory effect was not mediated by cAMP; this is in accord with the *in vitro* observation of Kawashima et al[6].

In human studies, Lee et al[7] found that

chronically elevated endogenous CT did not affect serum 1,25-$(OH)_2$D and 25-OHD concentrations in patients with medullary carcinoma of the thyroid. Emmertsen et al[2], on the other hand, reported that serum 1,25-$(OH)_2$D levels were increased and 25-OHD levels were decreased in medullary carcinoma of the thyroid. The reasons for the discrepancy between these two similar studies are not known. In our previous study high or normal plasma levels of 1,25-$(OH)_2$D before CT therapy were decreased after one month of therapy and remained normal thereafter in OI patients, and plasma 25-OHD values remained normal during CT therapy[9]. Our results indicate that the acute and chronic effects of CT on vitamin D metabolism may differ. The chronic effect of CT may be in accord with the observation of medullary carcinoma of the thyroid by Lee et al[7]. In this study, plasma 1,25-$(OH)_2$D concentrations were increased in one normal adult and one OI patient, and 25-OHD did not change. This study is different from our previous reports of the acute effect of CT on plasma 1,25-$(OH)_2$D in OI patients and from experimental animal studies, except those of Rasmussen et al[10]. These discrepancies may be due, in part, to differences in investigative conditions, although a reasonable comparison may be difficult to draw between human studies and animal studies. For instances, this study was performed in normal adults and one OI patient, whereas our previous study was done in six OI patients. The doses of CT injected might be small in this study. Animal studies were performed in vitamin D-deficient states with or without thyroparathyroidectomy[3,5,6,8,10], but human studies were performed in normal vitamin D states[2,7,9].

No consistent abnormalities of biochemical values were reported in OI during chronic administration of CT[9]. The acute effects of CT administration include not only increases in plasma cAMP and in urine Ca and P but also decreases in serum Ca and in %TRP[4,25]. Urinary excretion of cAMP has been reported to be unchanged or increased in acute studies of CT[1,4,15]. These findings are similar to those in this study except for plasma cAMP. In this study, plasma cAMP levels were decreased after CT injection. The effect of CT on plasma cAMP may be direct and not a consequence of PTH secretion due to hypocalcemia, since plasma cAMP increases after PTH injection in normal humans[1]. The exact mechanism of this change is not clear.

In any event, since there are still many different observations of acute and chronic effects of CT on vitamin D metabolism and cAMP, further studies are needed to clarify these problems.

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