Phosphaturic Effect of I.V. Administered Calcitonin in Man

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Synopsis

Porcine calcitonin (CT) was administered intravenously to 4 patients whose kidney function and plasma Ca and P levels were normal. A significant increase in urinary P excretion with an increase in urinary cyclic AMP (cAMP) excretion was observed. Plasma P and cAMP levels which were determined simultaneously did not show any significant change. Furthermore, plasma PTH levels determined by the radioimmunoassay revealed that the increased PTH levels provoked by the CT infusion was not responsible for an increase in urinary P or cAMP excretion, since no increase in plasma PTH levels was found during the period when an increase in urinary P or cAMP excretion was observed. In addition, the CT infusion caused an increase in urinary excretion of Ca, Na and Mg as well as P.

Based on these data, it can be concluded that CT administered intravenously acts directly on the kidney, resulting in an increase in urinary P excretion which could be mediated through cAMP in the kidney.

It is well known that calcitonin (CT) administration causes phosphaturia, but there is a controversy about the mechanism. In the rat, CT has been shown to increase the renal excretion of P in both intact and thyroparathyroidectomized animals (Kenny *et al.*, 1965; Milhaud *et al.*, 1966; Robinson *et al.*, 1966). However, in the dog this effect depended on the presence of the parathyroids (Pak *et al.*, 1970). Ardaillou *et al.* reported that a patient with hypoparathyroidism responded to porcine CT with a significant phosphaturia (Ardaillou *et al.*, 1968). Haas *et al.* infused porcine, human or salmon CT to 8 patients with untreated surgical hypoparathyroidism and documented that pharmacological doses of CT increased the renal

clearance of P, Ca, Na and Mg independently of the parathyroids (Haas *et al.*, 1972).

In the present study, porcine CT was infused to 3 patients with advanced breast cancer and a patient with Turner's syndrome whose renal function or plasma Ca and P levels were normal. Plasma Levels of P, Ca, PTH and porcine CT as well as urinary excretion of P, Ca and creatinine were determined to clarify whether the phosphaturic effect of the intravenously administered CT in man was due to a direct effect on the kidney or to the PTH secreted in response to the CT administration. In addition, urinary levels of Na, Mg and K were measured following the CT infusion to examine the CT effect on these electrolytes excretion from the kidney.

Furthermore, urinary excretion of adenosine 3',5'-monophosphate (cAMP) as well as plasma levels of cAMP was determined following the CT administration in order to

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study the role of the nucleotide in relation to phosphaturia.

Materials and Methods

Calcitonin; porcine calcitonin (CT), 73.4 MRC units per mg, was obtained from Armour Pharmaceutical Company, Ltd., Astboune, England.

Plasma PTH levels were determined by the radioimmunoassay as reported previously (Tanaka *et al.*, 1974). Calcium (Ca) and magnesium (Mg) were determined by atomic absorption spectrophotometry and phosphate (P) by the method of Goldenberg and Fernandez (Goldenberg and Fernandez, 1966).

Plasma and urinary levels of cAMP were determined by the protein binding method (Gilman, 1970). Binding protein was prepared from the rat liver according to the method of Kumon *et al.* (Kumon *et al.*, 1970). Fresh, heparinized blood was collected in ice-chilled tubes and the plasma was separated without delay by an refrigerated centrifuge. Cyclic AMP was extracted by adding one tenth volume of 50% TCA and excess TCA was removed by water saturated ether. Twenty to 100 times greater dilution was required to determine the cAMP levels in the urine samples.

Plasma levels of porcine CT were determined as follows. Antisera were obtained from rabbits immunized with porcine CT. The immunizing schedule was that 0.27 mg porcine CT (Armour) emulsified with complete Freund's adjuvant was injected subcutaneously every 2 weeks for 2 months. Booster immunization was added every 2 months and at the end of 6 months antisera were obtained. Highly purified porcine CT used for the iodination and the radioimmunoassay standard was kindly provided by Dr. J. W. Bastin of Armour Pharmaceutical Co. The iodination was performed based on the method of Hunter and Greenwood (Hunter and Greenwood, 1962). QUSO was used to separate ^{125}I -porcine CT from free ^{125}I (Tashjian, 1969). ^{125}I -porcine CT was repurified through Sephadex LH-20 column (Excel SM 300, 0.9×15 cm) with 0.1 M acetic acid prior to adding to the incubation mixture. Three peaks were usually observed and the middle peak was used. The specific activity was determined by the radioimmunoassay which was about $100 \mu\text{Ci}/\mu\text{g}$. The double antibody method was used to separate the bound from the free. The 10% fall of B/T was observed at the porcine CT concentration of 270 pg/ml. There was no cross reaction with salmon or bovine CT.

The subjects studied were 3 patients with advanced breast cancer and one patient with Turner's syndrome. Their renal function was normal and their plasma

levels of Ca and P were in a normal range. Two patients with advanced breast cancer, aged 49 and 54, and a patient with Turner's syndrome, aged 36, were given 80 MRC units of porcine CT. A patient with advanced breast cancer, aged 56, received 160 MRC units of porcine CT.

After their overnight fast, the subjects were given 250 ml of water to drink one hour prior to the CT infusion and every 2 hour till the end of the study in order to ensure adequate urine flow. Just prior to the study, a urethral catheter was inserted to collect urine samples.

Porcine CT dissolved in 150 ml of 5% glucose solution was infused to the subjects during the period of 30 min. Blood samples were collected before and every hour up to 5 hours following the CT infusion in 3 cases who had received 80 or 160 MRC units. In a case with advanced breast cancer who had received 80 MRC units, blood samples were collected before and 5, 20, 40, 60, 90 and 120 min to determine plasma levels of PTH and porcine CT following the CT infusion.

Urine samples were collected every 30 min before and following the CT infusion and every urine sample was designated by the time when to finish the urine collection. For example, the 60 min urine sample indicates the urine collected for 30 and 60 min after starting the CT infusion.

There were no serious side effects except that a case with advanced breast cancer who had received 80 MRC units complained of nausea during the infusion.

Results

Urinary P and cAMP excretion following the CT infusion (Fig. 1).

A significant increase in urinary P excretion was observed in all 3 cases who had received the CT infusion. A peak was at the 60 min urine sample in 2 cases. One of these was a patient with advanced breast cancer who had received 160 MRC units and the other was a patient with Turner's syndrome who had received 80 MRC units. In a case with advanced breast cancer who had received 80 MRC units, urinary P excretion was kept elevated from the 30 min to 5 hr urine sample.

A significant increase in urinary cAMP was also observed in all 3 cases who had received the CT infusion. In 2 cases whose peak value

Fig. 1. Urinary P and cyclic AMP excretion following the CT infusion.

of urinary P excretion was observed in the 60 min urine sample, urinary cAMP excretion showed a sharp peak at the 30 min urine sample in one who had received 160 MRC units and in the other who had received 80 MRC units urinary cAMP excretion was elevated at the 30 min, 60 min and 90 min urine samples. In a case who had received 80 MRC units and whose urinary P excretion was kept elevated from the 30 min to 5 hr urine samples, urinary cAMP excretion began to increase from the 30 min urine sample, showed a peak at the 2 hr urine sample and stayed high till the end of the study.

Plasma Ca, P, PTH and cAMP levels following the CT infusion (Fig. 2).

Plasma Ca and P levels did not show any

Fig. 2. Plasma levels of Ca, P, PTH and cyclic AMP following the CT infusion.

significant changes following the CT infusion. Plasma PTH levels following the CT infusion did not show any significant increase during the period when the increase in urinary P or cAMP excretion was observed. An increase in plasma PTH levels was found in a case who had received 160 MRC units 3 hr after the CT infusion, but it occurred only after the urinary P or cAMP excretion began to fall towards the basal level. The other case whose plasma PTH levels did not show any significant changes following the CT infusion was the one in which urinary excretion of P or cAMP was kept elevated till the end of the study.

Plasma cAMP levels did not show any reasonable increases which corresponded with the urinary cAMP excretion in response to the CT infusion.

Plasma PTH and porcine CT levels following the CT infusion (Fig. 3).

Plasma PTH and porcine CT levels as well as plasma Ca and P levels were depicted in Fig. 3 following the CT infusion in a patient with advanced breast cancer, who had received 80 MRC units. Although there were no significant changes in plasma Ca and P levels following the CT infusion, plasma levels of porcine CT were increased from the basal level of less than 0.05 ng/ml to 27.0 ng/ml 20 min after starting the CT infusion, which fell to 0.1 ng/ml at 120 min. Plasma PTH levels began to increase 60 min after the CT infusion had started.

Urinary excretion of Ca, Na and Mg following the CT infusion (Fig. 4).

A significant increase in urinary excretion of Ca, Na and Mg was observed following the

Fig. 3. Simultaneous determinations of plasma Ca, P, PTH and porcine CT levels in a patient received 80 MRC units porcine CT.

Fig. 4. Urinary excretion of Ca, Na and Mg following the CT infusion.

CT infusion. A peak value was found at the 30 min urine sample regarding these electrolytes. However, no increase in urinary K excretion was observed.

Discussion

It has been well established that CT lowers the plasma levels of Ca and P by inhibiting bone resorption, but the more studies are still required to clarify its possible action on the kidney (Hirsh and Munson, 1969).

In order to study the renal effects of CT in man, porcine CT was administered intravenously at a dose of 80–160 MRC units to human subjects and it was found that urinary P and cAMP excretion was increased simultaneously. Concomitant determinations of

plasma P and cAMP levels excluded the possibility that an increase in urinary P or cAMP excretion was secondary to an increase in plasma levels of these components. Furthermore, the determination of plasma PTH levels following the CT infusion revealed that the increased plasma PTH levels provoked by the CT infusion was not responsible for an increase in urinary P or cAMP excretion, since no increase in plasma PTH levels was found during the period when an increase in urinary P or cAMP was observed.

Therefore, it can be concluded that CT acts on the kidney directly resulting in an increase in urinary excretion of cAMP and in phosphaturia.

It is interesting to note that the cAMPuric effect of a maximal dose of the infused CT was far smaller than that caused by the PTH infusion in the presence of almost comparable stimulation of cAMP accumulation in the whole kidney *in vivo* (Kurokawa *et al.*, 1974). An increase in urinary excretion of cAMP induced in man by the CT infusion reported here was also much smaller than the tremendous increment of cAMP excretion known to be caused by PTH (Lewis *et al.*, 1967). However, there is no evidence enough to explain such difference yet.

CT was first shown to stimulate an accumulation of cAMP in the rat kidney homogenate (Murad *et al.*, 1970) and to increase the adenylyl cyclase activity in the separated membranes of rat kidney (Marx *et al.*, 1972). A recent study demonstrated that the CT infusion to thyro-parathyroidectomized rats induced a marked increase in cAMP accumulation in the whole kidney *in vivo* and enhanced urinary excretion of this nucleotide (Kurokawa *et al.*, 1974). Although substantial evidences including this report that CT has a direct effect on cAMP metabolism in the renal tissue have been accumulated, it can't be decided whether cAMP is a key metabolite in the renal action of CT. The micropuncture study by Goldberg *et al.* demonstrated that dibutyryl cAMP inhibited the proximal tubu-

lar function and lead to diuresis of Na, P and Ca (Goldberg *et al.*, 1972). Recently the qualitative similarity between the effect of CT and that of exogenously administered cAMP on the kidney was emphasized by Rasmussen *et al.* (Rasmussen *et al.*, 1974).

Therefore, it is highly possible that cAMP is involved in the renal effect of CT as a second messenger as shown in many occasions of the other polypeptide hormones.

It has been reported that CT causes an increase in urinary excretion of electrolytes such as Ca, Na or Mg in addition to P (Ardaillou *et al.*, 1968; Haas *et al.*, 1972; Bijvoet *et al.*, 1972). According to our data presented here, urinary excretion of Ca, Na and Mg was found to increase significantly in response to the CT infusion. Since no simultaneous increase in plasma levels of these electrolytes was found and the effect was observed at the time when plasma porcine CT levels were elevated, it can be said that CT acted on the kidney, resulting in an increase in urinary excretion of these electrolytes.

It can be concluded that CT has a direct effect on the kidney, and causes an increase in urinary excretion of P as well as Ca, Na and Mg. These renal effects of CT might be mediated by cAMP, but the exact mechanism remains to be elucidated.

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