Extracellular cyclic AMP levels in osteomalacia

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Abstract. Plasma and urinary cyclic AMP were measured in a 62-year-old man with severe osteomalacia due to a gluten-induced enteropathy. Before therapy, plasma cyclic AMP was normal and both urinary and nephrogenous cyclic AMP were markedly increased. A calcium infusion acutely diminished the elevated iPTH and urinary cyclic AMP levels. Following a bolus infusion of PTE, there was no phosphaturia despite a somewhat exaggerated increase in cyclic AMP excretion. With appropriate therapy, serum calcium, phosphorus, alkaline phosphatase, iPTH and 25-OHDs and urinary and nephrogenous cyclic AMP gradually returned to normal. The response to PTE infusion after normalization of serum electrolytes, iPTH, and 25-OHDs levels was similar to that seen before therapy.

These results demonstrate that non-azotaemic malabsorptive osteomalacia is associated with elevations in urinary and nephrogenous cyclic AMP which gradually fall to normal with therapy. In contrast to studies in the vitamin D deficient rat, the maximal urinary cyclic AMP response to PTE is not diminished in the human with malabsorptive osteomalacia. The lack of a phosphaturic response to PTE despite a normal increase in urinary cyclic AMP excretion, as seen in our patient, is similar to that described in some patients with pseudohypoparathyroidism, suggesting that the response is not specific for the latter disorder. Finally, the data suggest that endogenous PTH does not modulate its renal receptors, at least in terms of cyclic AMP generation.

It is generally accepted that vitamin D plays a major role in the action of parathyroid hormone (PTH) in bone, whereas in the kidney, PTH can exert its usual effects in the absence of vitamin D (Rasmussen et al. 1963; Arnaud et al. 1966). In keeping with the critical effects of vitamin D on calcium metabolism, a deficiency in this substance is typically associated with skeletal resistance to PTH (Steendijk 1964) and the development of hyperparathyroidism (Arnaud et al. 1972; Joffe et al. 1972). Evidence of an increase in PTH secretion is supported by recent studies in which children with rickets had significant elevations in urinary cyclic AMP (cAMP) excretion (Sovik et al. 1976; Vainsel et al. 1976). Forte et al. (1976) found that in vitamin D deficient rats the administration of PTH was associated with a diminished response in terms of phosphate and cAMP excretion and renal cell adenylate cyclase activity. They suggested that high levels of endogenous PTH may have reduced renal receptors blunting any effect from exogenous PTH. In this laboratory, the cAMP and phosphaturic responses to an infusion of parathyroid extract (PTE) in normal subjects and in patients with primary hyperparathyroidism and hypoparathyroidism were found to be comparable (Tucci et al. 1979), suggesting that the renal cAMP response to a bolus of PTE is not affected by the level of serum calcium, phosphorus, or endogenous PTH.

Recently, similar studies were performed in a man suffering from celiac disease, severe osteomalacia, and secondary hyperparathyroidism. The results of these studies including the measurement of extracellular cyclic nucleotide levels before and
after infusions of PTE, prior to and subsequent to appropriate therapy form the substance of this report.

Case Study

A 62-year-old male, retired steel worker, entered the Roger Williams General Hospital in September, 1976, with a 2-year history of progressive pain and discomfort primarily affecting his lower extremities, back and ribcage. He also noted progressive and generalized weakness and walked with a wobbly gait. He had the classical symptoms of malabsorption syndrome and had lost 9 kg during the preceding year.

On physical examination muscle bulk appeared somewhat diminished throughout. Pain was elicited with pressure over the ribcage and shins. His gait was unstable but equilibrium and coordination were intact. Strength was diminished in the pelvic girdle and proximal thigh muscles. Chvostek's sign was weakly positive, and Trousseau's sign was absent.

Serum calcium varied from 6.7 to 7.6 mg/100 ml (normal 8.5 to 10.5). Serum ionized calcium was 3.8 mg/100 ml (normal 3.8 to 4.5), and serum phosphorus ranged from 2.4 to 3 mg/100 ml (normal 2.5 to 4.5). Alkaline phosphatase levels were persistently elevated and on fractionation attributable to bone. Serum hydroxycholecalciferol (25-OH) was undetectable and serum iPTH was 108 μEq/ml (normal < 90). Endogenous creatinine clearances varied from 81 to 128 ml/min, and phosphorus clearances from 13 to 26 ml/min. Tubular re-absorption of phosphorus (TRP) varied from 86 to 90%, and maximal tubular re-absorption as a function of glomerular filtration (TmP/GF) from 2.1 to 2.45 mg/100 ml GF (normal 2.5 to 4.2). Urinary calcium excretion was diminished at less than 50 mg/day. Serum magnesium was 1.5 mg/100 ml (normal 1.8 to 2.9), while urinary magnesium excretion was normal. An infusion of magnesium resulted in an increased retention of 89%. Ninety-four per cent of a load of infused calcium was retained.

Fig. 1.
Serum calcium and phosphorus levels and urinary calcium, phosphorus, and cyclic AMP/ct ratios prior to and during therapy. The normal range of values is represented in the shaded areas.
During the infusion, serum calcium rose 1.9 mg/100 ml, iPTH fell to normal and thyrocalcitonin was undetectable.

Serum carotene was 12 µg/100 ml (normal 16 to 33). Seventy-two hour stool fat content was 41.3 g/day (normal <15 g). Gastrointestinal X-rays revealed increased folds in the gastric fundus and second portion of the duodenum, dilated small bowel loops and rapid intestinal transit. A small bowel biopsy revealed flat atrophic mucosa. Bone X-rays revealed generalized demineralization, coarse trabeculations, and fractures without healing calluses. Biopsy of the anterior iliac crest revealed markedly increased osteoid matrix. Chemical changes observed prior to and during therapy are depicted in Fig. 1. Within 3 months of therapy, serum calcium and phosphorus were normal. Alkaline phosphatase rose from 354 mU/ml to its peak level of 633 mU/ml by the 4th month of therapy and then began to fall toward normal. Urinary calcium excretion was low while urinary phosphorus excretion tended to reflect the dietary intake and serum phosphorus levels.

After initial metabolic studies were completed, he was placed on a gluten-free diet, supplemented with 750 mg of elemental calcium daily and vitamin D5 50,000 units twice weekly. He improved progressively, noted increasing strength, intestinal symptoms abated, and the previously noted fractures disappeared.

Materials and Methods

Metabolic studies were performed on our Clinical Investigation Unit. The patient was maintained on a constant diet containing 150 mg of calcium and 500 mg of phosphorus per day. Twenty-four hour urine specimens were collected under ice in containers to which 15 ml of 6 N HCl were added. Hourly urine specimens were immediately frozen without preservative. Serum calcium, phosphorus, and creatinine were determined on a multi-channel Technicon Autanalyzer. Serum ionized calcium was measured by an ionselective electrode (Orion SS-20). Urinary calcium and serum and urinary magnesium were determined by atomic absorption. PTH and thyrocalcitonin levels were determined by immunoassay and serum 25-OHDS by competitive binding assay1. Plasma cAMP was determined by a radioimmunoassay technique with kits supplied by the Schwartz Mann Company. Urinary cAMP was determined by a modification of the technique of Gilman (1970) as previously described. Nephrogenous cAMP (NcAMP) is expressed as a percentage of total urinary cAMP (Babka et al. 1976), and as a function of the glomerular filtration rate (Broadus et al. 1977) as follows:

\[
\text{NcAMP} = ucAMP \times \frac{\text{Ccr}}{\text{Ccr} \times 100}
\]

The maximal tubular re-absorption of phosphorus as a function of glomerular filtration was calculated using Bijvoet's monogram (Bijvoet & Van der Sluys Veer 1972).

The magnesium retention test was performed as described by Thoren (1963). The retention rate of infused calcium was calculated as described by Hass et al. (1963), following the infusion of 10 mg of elemental calcium/kg body weight over a 4-h period. Parathyroid stimulation test was performed after an overnight fast. Two hundred units of bovine PTE (Eli Lilly Company) was infused over 3 min beginning at 09:00 h, and hourly urine specimens were collected before and after the infusion and together with timed serum specimens were analyzed for their creatinine, phosphorus, and cAMP contents.

Results

Urinary cAMP excretion was markedly increased (see Table 1). Daily excretion ranged from 16.36 to 21.09 µmol/24 h and cAMP/creatinine ratios from 22.46 to 27.40 µmol/g. Plasma cAMP levels were normal. Accordingly, NcAMP was increased and accounted for 84 to 89.5% of the total excreted. NcAMP varied from 8.50 to 13.73 nmol/100 ml GF. Following the infusion of calcium, there was a 42% fall in urinary cAMP excretion whether expressed in terms of creatinine or as a function of GFR.

By the 4th month of therapy, urinary cAMP excretion had fallen but was still elevated with excretion levels of 10.7 to 11.01 µmol/24 h and ratios of 7.78 to 7.98 µmol/g. By the 7th month of therapy, cAMP excretion was normal. At this time,

<table>
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<tr>
<th>Table 1.</th>
<th>Extracellular cyclic AMP levels in osteomalacia.</th>
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<td></td>
<td>Before Rx</td>
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<tr>
<td><strong>Urinary cAMP</strong></td>
<td></td>
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<tr>
<td>µmol/24 h (3.43–8.21)*</td>
<td>16.36–21.09</td>
</tr>
<tr>
<td>µmol/g Cr (1.98–5.14)</td>
<td>22.46–27.40</td>
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<tr>
<td><strong>Plasma cAMP</strong></td>
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<tr>
<td>pmol/ml (12.8–30.7)</td>
<td>26.2</td>
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<tr>
<td><strong>Nephrogenous cAMP</strong></td>
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<tr>
<td>% urinary cAMP (28–62)</td>
<td>87–89%</td>
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<tr>
<td>nmol/100 ml GF (0.22–3.25)</td>
<td>8.50–13.73</td>
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* Normal range of values.

1 Nichols Institute, San Pedro, California.
Urinary P/cr and cyclic AMP/cr ratios before and after infusion of 200 units of parathyroid extract.

Fig. 2.

Serum PTH was also normal at 17 μEq/ml. With therapy, TRP varied from 86 to 90% and TmP/GF rose to normal and varied from 4.49 to 2.52 mg/100 ml.

Before therapy, an infusion of PTE resulted in an equivocal phosphaturic response while urinary cAMP rose from 17.17 nmol/min and 16.06 μmol/g creatinine to 311.83 nmol/min and 512.75 μmol/g, respectively (Fig. 2). The urinary response was somewhat exaggerated when compared to that seen in a group of normal subjects in whom urinary cAMP rose from 4.16 to 180 nmol/min and 4.97 to 175 μmol/g creatinine (Tucci et al. 1979). Plasma cAMP levels also rose from a control level of 28.4 to 65.1 pmol/ml 1 h after the infusion. nCAMP after the infusion of PTE rose to a maximum of 97.5% of the total urinary cAMP excreted.

Therapy had no apparent effect on the response to an infusion of PTE. Again, there was an equivocal phosphaturic response and a normal or exaggerated increase in plasma and urinary cAMP levels. Urinary cAMP excretion of 5.00 nmol/min increased to 300.50 nmol/min and cAMP/creatinine ratio of 4.53 rose to 431.01 μmol/g.
Discussion

The case herein presented had the typical features of malabsorption and osteomalacia. The diagnosis of celiac disease was made on the basis of biochemical abnormalities, small bowel biopsy, and the clinical response to a gluten-free diet. The diagnosis of osteomalacia was also firmly established on both clinical and laboratory grounds. Vitamin D deficiency was confirmed by undetectable serum 25-OH-D$_3$ levels. This deficiency was almost certainly caused by malabsorption of this vitamin, but impairment in enterohepatic circulation (Haddad 1977) and hepatic injury that may occur in adult celiac disease (Hagander et al. 1977) could also have played a role.

The low serum magnesium and increased retention of an iv load of magnesium were consistent with magnesium depletion. This was not sufficient, however, to prevent the elevation in endogenous PTH (Suh et al. 1973; Anast et al. 1976) nor to protect the patient’s skeletal system from developing osteomalacic changes as reported by Muldowney et al. (1970).

The elevated serum iPTH and urinary cAMP were consistent with a hyperparathyroid state. Elevations in serum iPTH have been well documented in children (Arnaud et al. 1972; Fisher et al. 1973) and adults (Joffe et al. 1972; Stanbury et al. 1975) with vitamin D deficiency. In rachitic children, marked increases in cAMP excretion have been reported (Sovik et al. 1976; Vainsel et al. 1976). In the few adults studied with non-azotemic vitamin D deficient osteomalacia, however, only small increases in urinary cAMP have been reported (Aurbach et al. 1970; Bernard et al. 1976; Schmidt-Gayk et al. 1977), and little or no clinical information provided. The urinary cAMP values observed in our patient are the highest thus far reported in osteomalacia, perhaps correlating with the advanced stage of bone involvement (Preece et al. 1973; Stanbury et al. 1975). Indeed, the cAMP values in this patient were greater than those observed in 21 patients with proven hyperparathyroidism (7.68 ± 0.87 μmoles/g)$^2$ despite comparable iPTH levels (Tucci et al. 1979). Since plasma cAMP levels were normal, the increases in urinary cAMP are attributable to the nephrogenous component which accounted for 80% of the cAMP excreted.

Tomlinson et al. (1976b) have reported a diminished plasma cAMP response to a bolus of PTE in 2 of 6 patients with hyperparathyroidism whose iPTH levels were very high and in subjects given a continuous infusion or repeated boluses of PTH. These investigators suggested saturation of renal receptor sites as an explanation for the diminished response to PTE (Tomlinson et al. 1976a). Also, in vitamin D deficient rats, Forte et al. (1976) have demonstrated diminished renal cAMP responses to PTE administration and suggested high PTH levels decrease renal cell receptors. However, other investigators have found a normal cAMP response to PTE in vitamin D deficient rats (Nagata & Rasmussen 1968). Interestingly, vLilienfeld-Toal et al. (1978) recently noted that several patients with various gastrointestinal diseases had high baseline cAMP excretion and supranormal cAMP response to PTE administration. In studies from this laboratory, the acute cAMP response to PTE in 21 patients with surgically proven primary hyperparathyroidism were found to be comparable to the responses in normal subjects and in patients with hypoparathyroidism (Tucci et al. 1979). The osteomalacic patient herein presented had a cAMP response to PTE which was as great if not greater than the responses found in these groups of subjects. A similar response was seen after therapy. These findings suggest that serum levels of calcium, phosphorus, and iPTH and, now, vitamin D probably have no significant effect on the maximal renal cAMP response to PTE in man.

Despite the cAMP response, our patient did not have a phosphaturic response to PTE. This pattern is similar to that previously described in some patients with pseudohypoparathyroidism (Dreznner et al. 1973) and suggests that the so-called ‘Type II’ response may be non-specific. Also, it is now clear that the phosphaturic response to PTE originally described by Ellsworth & Howard (1934) is not a consistent finding (Aurbach et al. 1970) and may not be seen in normal subjects (Chase et al. 1969; Tomlinson et al. 1974). The significance, then, of a normal cAMP and a blunted phosphaturic response is unclear.

The present studies demonstrate that non-azotemic malabsorptive osteomalacia can be characterized by marked elevations in urinary and NCAMP. The acute reduction of cAMP excretion with an infusion of calcium is consistent with suppressible parathyroid gland activity. The gradual return of urinary cAMP to normal with appropri-
ate therapy and normalization of serum calcium suggest that cAMP measurements may be of value not only in the diagnosis of osteomalacia but also in the assessment of the response to therapy. The cAMP responses to PTE in this patient parallel our observations in patients with primary hyperparathyroidism and hypoparathyroidism and suggest that endogenous PTH does not modulate its renal receptors.

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References


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