Phosphorus reduces renal receptivity for parathyroid hormone in rats

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Abstract: Changes in kidney calcium concentration and secreted parathyroid hormone were studied in weanling male rats (n = 12) fed diets containing either 0.5% (n = 6) or 1.5% (n = 6) total phosphorus. Calcium and phosphorus concentrations in the kidney of rats fed a high-phosphorus diet were markedly greater than those in rats fed a control diet. In addition, urinary excretion of phosphorus increased gradually after administration of a high-phosphorus diet, but there was no similar tendency of phosphorus/creatinine excretion, which decreased gradually from the starting day of feeding to the end of the feeding period. The high-phosphorus diet also produced greater serum parathyroid hormone (PTH) without urinary cyclic adenosine monophosphate (cAMP) excretion stimulated by PTH. The mean values of serum 1,25-dihydroxycholecalciferol (1,25(OH)2D3) concentrations were significantly increased 1 h after injection of 2.77 μg rat PTH(1-34) in all rats. However, in rats fed a high-phosphorus diet, the rise of serum 1,25(OH)2D stimulated by exogenous PTH was lower than that in rats fed a control diet.

Key words: phosphorus, nephrocalcinosis, parathyroid hormone, cAMP, 1,25(OH)2D3

Introduction

Nephrocalcinosis is a disorder involving intracellular and intratubular deposition of calcium phosphate [1]. It can be provoked by a variety of factors, which include high phosphorus consumption. Clark and Rivera-Cordero [2] showed that nutritional secondary hyperparathyroidism was induced by a high-phosphorus diet. Parathyroid hormone (PTH) regulates the concentration of plasma calcium in the circulation. Normally, PTH acts by decreasing urinary calcium in the kidney, and stimulates calcium release on bone. PTH is also a prominent regulator of renal vitamin D metabolism. It has been shown to stimulate 1,25-dihydroxycholecalciferol (1,25(OH)2D3) synthesis [3-5] on the kidney through a mechanism involving cyclic adenosine monophosphate (cAMP). Since the action of PTH on the kidney was shown to be associated with the accumulation of cAMP in the cell and an increase in the urinary excretion of cAMP [6], the measurement of urinary excretion of cAMP in effect constitutes an in vivo bioassay of circulating bioactive PTH. Previous studies have shown that a high-phosphorus diet induces extensive PTH-dependent nephrocalcinosis [2,7]. Whether the stimulation of PTH by high-phosphorus supplementation had a genuine effect on calcified kidney is unknown. The aim of the present study was to explore alterations of PTH secretion in rats supplemented with a high-phosphorus diet. Moreover, renal receptivity for PTH action were examined using urinary cAMP excretion and other metabolites of PTH action.

Materials and methods

Experimental procedures

The 4-week-old weanling male Wistar rats (n = 12) were obtained from Clea Japan (Tokyo, Japan) and housed individually in stainless steel cages in a room maintained at 22°C with a 12-h light-dark cycle. The study was approved by the Tokyo University of Agriculture Animal Use Committee, and the animals were maintained in accordance with guidelines for the care and use of laboratory animals of Tokyo University of Agriculture. For 1 week, the rats were fed a specially formulated control diet (Table 1) containing 0.5% phosphorus. Starting on day 0, the rats were subsequently divided into two groups of six each. The first group was
fed a control diet, the other one received the high-phosphorus diet (Table 1) containing 1.5% phosphorus added as K₂PO₄. Food and distilled water were given freely, and body weight was recorded daily. From days 0–3, 6–10, and 13–17, 24-h urine samples were collected in the metabolic cage. On day 21 of the experimental period, after 600 µl of blood had been collected, rats were given an injection of 2.77 µg rat PTH(1–34) (Sigma Chemical, St. Louis, MO, USA). Then, 1 h later, the rats were killed and kidney and blood samples were collected for analysis.

Analytical methods

Rat Kidneys were freed of fat and the renal capsule, and the “wet weight” was immediately measured. The whole kidneys were dried overnight at 100 °C, weighed, and then ashed for 48 h at 550 °C. After measurement of kidney ash weight, the minerals were extracted for analysis by demineralization with 1 mol/l HCl solution. Urinary samples were diluted with H₂O to adjust the volume constantly, ashed, and then demineralized with a 1 mol/l HCl solution. The calcium and magnesium content of the kidney, urine, and serum were analyzed by atomic absorption spectrophotometry (Shimadzu AA 640-13) according to the method of Gimblet et al. [8]. Phosphorus was analyzed colorimetrically according to the method of Gomori [9]. Urinary creatinine was analyzed by the Jaffe reaction, as described by Lustgarten and Wenk [10]. Assay of serum parathyroid hormone was accomplished with a radioimmunoassay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). This method is a two-site immunoradiometric assay (IRMA) using two different goat antibodies to measure both intact and N-terminal PTH of rat. The 1,25(OH)₂D₃ was purified from the serum sample by passing it through a reverse-phase chromatography column C₁₈ (Sep-Pac, Waters Associates, Milford, MA, USA). Lipid extraction of the serum samples were applied to the C₁₈ column and eluted by water, 70 (v/v)% methanol in water, 10 (v/v)% methylene chloride in n-hexane, 1 (v/v)% isopropanol in n-hexane, and 5 (v/v)% isopropanol in n-hexane. 1,25(OH)₂D₃ fractions were eluted by 5 (v/v)% isopropanol in n-hexane. The active metabolite was quantified by radioimmunoassay [11], carried out with tritiated 1,25(OH)₂D₃ (Radiochemical Centre, Amersham, UK) as the radioactive tracer and calf thymus receptor for 1,25(OH)₂D₃ (Yamas Shoyu, Tokyo, Japan) as the binding protein. Urinary samples were diluted with 100 volumes of distilled water as a sample solution for assay of cAMP and creatinine. cAMP was determined by radioimmunoassay [12] using a cAMP assay system (Radiochemical Centre).

Statistical analysis

The data were presented as the means ± SEM for a group of six rats. One-way analysis of variance (ANOVA) was used to analyze the data, and a P value of less than 0.05 between the control and high-phosphorus group was considered significant [13].

Results

Growth. The final body weights of the rats fed the control diet were significantly higher than those of the rats fed the high-phosphorus diet.

Kidney calcium contents. The effects of dietary concentration of phosphorus on kidney calcification are shown in Table 2. The kidney calcium concentration was 0.25 ± 0.02 mmol/100 g dry weight in the rats fed the control diet. However, increasing the dietary phosphorus concentration from 0.5% to 1.5% increased renal calcium, and more than 1000 times higher values were observed in the high-phosphorus group than in the control group. Also, the kidney magnesium and phosphorus content was significantly higher in the high-phosphorus group than in the control group. The kidney dry weight was also greater in rats fed a high-phosphorus diet.

Table 1. Composition of experimental diets

<table>
<thead>
<tr>
<th>Dietary P level</th>
<th>Control 0.5% (g/100 g)</th>
<th>High-phosphorus 1.5% (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk casein</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mineralsb</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Vitaminsc</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>K₂PO₄</td>
<td>1.9</td>
<td>7.2</td>
</tr>
<tr>
<td>Corn starch</td>
<td>71.0</td>
<td>65.7</td>
</tr>
</tbody>
</table>

a Dry matter basis.

b Containing (%): NaCl, 53.71; Fe(C₃H₆O₇)₂+H₂O, 0.954; ZnCl₂, 0.031; MnSO₄·H₂O, 0.185; CuSO₄·H₂O, 0.239; KI, 0.001; CaCO₃, 44.873; (NH₄)₆Mo₇O₂₄·H₂O, 0.004; MgSO₄·H₂O, 9.98.

c Containing per 1 g: retinol palmitate 2500 IU; thiamine sulfate 1 mg; riboflavin 1.5 mg; pyridoxine HCl 1 mg; cyanocobalamin 1 µg; ascorbic acid 37.5 mg; ergocalciferol 200 IU; tocopherol acetate 1.1 mg; calcium pantothenate 5 mg; nicotinic amide 10 mg; folic acid 0.5 mg.