The effect of a high phosphorus diet on the parathyroid cell cycle

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Abstract

High extracellular phosphorus directly increases parathyroid hormone (PTH) secretion and gene transcription. The present study evaluates the effect of a high phosphorus diet on the parathyroid cell cycle in rats with normal renal function. Rats were divided into two groups, receiving either a high phosphorus diet (HPD, \( P = 1.2\% \)) or a normal phosphorus diet (NPD, \( P = 0.6\% \)). The dietary calcium content was 0.6% in both diets. Rats were pair fed and sacrificed on days 0, 1, 5, 10 and 15 after initiation of the diet. The parathyroid glands were removed and parathyroid cells dispersed for evaluation of cell cycle and apoptosis by flow cytometry. Serum calcium, phosphorus, PTH and paracalcitriol were measured. As compared with NPD, the ingestion of a HPD resulted in an increased number of cells in the S phase of the cell cycle from day 1 to 10 (1.2 ± 0.09% vs 0.6 ± 0.04% for day 1, 1.2 ± 0.11% vs 0.6 ± 0.06% for day 5 and 1.0 ± 0.09% vs 0.5 ± 0.04% for day 10, \( P < 0.01 \)). By day 15, the percentage of cells in the S phase in NPD and HPD were not different. In the rats fed the HPD, serum PTH increased significantly from day 5 through 15 (\( P < 0.01 \)). Parathyroid cell apoptosis was minimal and unaffected by the diet. At day 15, the parathyroid gland size in HPD was increased by 27% as compared with NPD (\( P < 0.05 \)). This increase should be attributed to cell proliferation since parathyroid cell size remained unchanged. Serum calcitriol and calcium were not significantly different in the two groups. In HPD, an increase in serum phosphorus was observed only on day 1. The results show that an HPD results in the stimulation of the parathyroid cell cycle independently of changes in calcium and calcitriol.

Key words: apoptosis; calcium; cell cycle; parathyroid hormone; parathyroid hyperplasia; phosphorus

Introduction

The accumulation of phosphorus due to a decrease in renal function is an important factor in the pathogenesis of secondary hyperparathyroidism [1]. Phosphorus retention promotes secondary hyperparathyroidism through an inhibition of calcitriol synthesis [2,3] and the induction of hypocalcaemia [4]. Both hypocalcaemia and calcitriol deficiency have been shown to increase the synthesis of parathyroid hormone (PTH) mRNA and induce parathyroid cell proliferation [5–7]. Recent in vitro and in vivo studies have demonstrated a direct effect of phosphorus on PTH secretion and synthesis [8–11]. The effect of phosphorus on parathyroid cell proliferation has been addressed recently by Naveh-Many et al., who showed that in uraemic rats fed a low phosphorus diet (LPD, \( P = 0.02\% \)), the parathyroid cell cycle was markedly inhibited [7]; however, in these rats the LPD produced hypercalcaemia which may decrease parathyroid cell proliferation. Parathyroid cell apoptosis was minimal and unaffected by the diet. At day 15, the parathyroid gland size in HPD was increased by 27% as compared with NPD (\( P < 0.05 \)). This increase should be attributed to cell proliferation since parathyroid cell size remained unchanged. Serum calcitriol and calcium were not significantly different in the two groups. In HPD, an increase in serum phosphorus was observed only on day 1. The results show that an HPD results in the stimulation of the parathyroid cell cycle independently of changes in calcium and calcitriol.

Subjects and methods

Animals and diets

Wistar rats weighing 200 g were divided into two groups: one was fed on HPD (\( P = 1.2\% \)), and a second group received

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Gland sizing

Three parathyroid glands were used to evaluate gland size. Assuming an elliptical shape, the parathyroid gland volume was derived from the length of the major axis (a) and the minor axis (b) at the maximal diameter section. The estimated volume was: $V = \frac{4}{3} \pi ab^2$.

Biochemical determinations

Serum ionized calcium was measured with a Selective Calcium Electrode (CIBA CORNING 634Ca/pH analyser). Serum phosphorus was measured by a colorimetric method using a specific kit (Sigma Diagnostics, MO). Serum PTH was measured by a specific immunoradiometric assay (IRMA) (Nichols Institute, San Jose Capistrano, CA), with an intra- and inter-assay coefficient of variation of 5.7 and 6.2% respectively. Serum calcitriol was measured with a radioreceptor assay (Nichols Institute), with an intra- and inter-assay coefficient of variation of 10.6 and 15.3% respectively.

Flow cytometry

Parathyroid cell cycle, apoptosis and size were evaluated by flow cytometry. Prior to flow cytometry measurements, cells were isolated by mechanical dispersion without the use of enzymes, under an inverted microscope ($10 \times$) and with the help of sharp Dupont forceps. This was followed by gentle pipetting. To maintain a high cell concentration, all these manipulations were performed in a small volume (50 $\mu$l) of phosphate-buffered saline (PBS).

The cell cycle was analysed in whole cells by a modification of the method described by Vindelov and Christensen for isolated nuclei [14]. Briefly, in a buffer containing the non-ionic detergent Nonidet-P40, cells were treated with DNase-free RNase to prevent dye binding to double-stranded RNA; then cells were stained with propidium iodide. A total of 15000 cells were acquired by the flow cytometer (FACScan, Becton-Dickinson, San Juan CA). The CELLFIT software (Becton-Dickinson) was used for data acquisition and analysis. Cell debris and clumps were removed from analysis by both groups is shown in Table 1 and Figure 1B. FL2W-FL2A parameters gating. The percentage of cells in each phase of the cell cycle was calculated via the rectangle fitting model (RFIT) program. The same samples prepared for cell cycle were collected and analysed by the flow cytometer with the LYSIS II software. Apoptotic cells were identified as hypodiploid DNA cells falling near the diploid peak. To assess changes in cell size, we used the Forward Scatter Parameter (LYSYS II software), comparing the channel where the peak of diploid cell was located.

Discussion

This study shows that in normal rats a HPD stimulates the parathyroid cells to progress to the S phase of the cell cycle in association with an increase in serum PTH. Serum calcium and calcitriol were not modified during the 15 day period of HPD; the serum phosphorus was moderately increased only in day 1 of the HPD. The increase in the percentage of cells in the S phase was observed as early as 24 h after initiation of the HPD and by day 15 the cell proliferation became normal.
Table 1. Biochemical and flow cytometry data

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
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<tbody>
<tr>
<td>S phase (% cells)</td>
<td></td>
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<td></td>
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<tr>
<td>NPD</td>
<td>0.5 ± 0.07</td>
<td>0.6 ± 0.04</td>
<td>0.6 ± 0.06</td>
<td>0.5 ± 0.04</td>
<td>0.5 ± 0.07</td>
</tr>
<tr>
<td>HPD</td>
<td>0.5 ± 0.07</td>
<td>1.2 ± 0.09*</td>
<td>1.2 ± 0.11*</td>
<td>1.0 ± 0.09*</td>
<td>0.7 ± 0.08</td>
</tr>
<tr>
<td>Apoptosis (% cells)</td>
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<td></td>
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<tr>
<td>NPD</td>
<td>0.08 ± 0.01</td>
<td>0.09 ± 0.03</td>
<td>0.06 ± 0.02</td>
<td>0.07 ± 0.02</td>
<td>0.07 ± 0.01</td>
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<tr>
<td>HPD</td>
<td>0.07 ± 0.01</td>
<td>0.05 ± 0.02</td>
<td>0.04 ± 0.03</td>
<td>0.06 ± 0.01</td>
<td>0.09 ± 0.02</td>
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<td>PTH (pg/ml)</td>
<td></td>
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<tr>
<td>NPD</td>
<td>51.1 ± 5.3</td>
<td>57.5 ± 7.1</td>
<td>60.8 ± 10.2</td>
<td>50.4 ± 10.8</td>
<td>52.3 ± 6.6</td>
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<tr>
<td>HPD</td>
<td>54.1 ± 7.2</td>
<td>67.6 ± 9.4</td>
<td>89.6 ± 11.3*</td>
<td>123.8 ± 13.2*</td>
<td>114.6 ± 15.3*</td>
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<td>ICa (mM)</td>
<td></td>
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<tr>
<td>NPD</td>
<td>1.27 ± 0.05</td>
<td>1.23 ± 0.04</td>
<td>1.20 ± 0.04</td>
<td>1.28 ± 0.04</td>
<td>1.27 ± 0.01</td>
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<tr>
<td>HPD</td>
<td>1.28 ± 0.02</td>
<td>1.17 ± 0.02</td>
<td>1.24 ± 0.02</td>
<td>1.25 ± 0.01</td>
<td>1.25 ± 0.02</td>
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<tr>
<td>Calcitriol (pg/ml)</td>
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<tr>
<td>NPD</td>
<td>20.5 ± 2.3</td>
<td>22.7 ± 3.0</td>
<td>22.8 ± 4.6</td>
<td>18.9 ± 2.7</td>
<td>21.0 ± 2.8</td>
</tr>
<tr>
<td>HPD</td>
<td>21.1 ± 1.2</td>
<td>16.8 ± 3.4</td>
<td>24.3 ± 5.4</td>
<td>20.0 ± 3.9</td>
<td>18.6 ± 3.5</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
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<tr>
<td>NPD</td>
<td>8.0 ± 1.0</td>
<td>8.1 ± 0.3</td>
<td>8.6 ± 0.3</td>
<td>8.6 ± 0.3</td>
<td>8.3 ± 0.5</td>
</tr>
<tr>
<td>HPD</td>
<td>8.2 ± 0.3</td>
<td>9.1 ± 0.6#</td>
<td>9.0 ± 0.6</td>
<td>9.1 ± 0.5</td>
<td>8.6 ± 0.2</td>
</tr>
</tbody>
</table>

All the results presented are means ± SE. 

#P < 0.05 vs NPD; *P < 0.01 vs NPD.

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these results suggest that the parathyroid cell cycle reached a new steady state once the parathyroid gland was enlarged.

Other authors have shown that in uraemic rats, a HPD (P = 0.8%) produced a significant enlargement of parathyroid glands even by 2 days after the induction of uraemia [11,15]. In normal rats, dietary phosphorus as high as 3.4% induced a 3-fold increase in cell birth rate; however in this latter report, the HPD was associated with a decrease in serum calcium and an increase in serum calcitriol [13].

In rats on the HPD, we observed an increase in parathyroid cell proliferation from day 5 to 10 despite no elevation in serum phosphorus. The fact that serum phosphorus did not increase does not exclude the possibility of an increase in the body burden of phosphorus resulting from the HPD; the phosphorus accumulation may be responsible for the increase in parathyroid cell proliferation. In addition, a high phosphorus meal can produce a transient change in serum phosphorus [9] which may directly affect parathyroid cell proliferation.

The fact that in rats fed an HPD the serum phosphorus remained at normal levels from days 5 through 15 can be explained by the elevation in PTH. We have shown previously that in rats the serum phosphorus can be maintained in the normal range despite a HPD if there is a concomitant increase in PTH [3]; this is what the classical ‘trade-off’ model has proposed [16]: parathyroid gland growth is being stimulated by the need to maintain a normal serum phosphorus and calcium. To achieve this increase in PTH, a larger number of cells secreting PTH may be required; our data show that the HPD produced an elevation of serum PTH which was subsequent to the increase in parathyroid cell proliferation (Figure 1A and B). The HPD increased parathyroid cell proliferation without an apparent modification in the very low (near absent)
number of apoptotic cells. After 15 days on the HPD, the parathyroid gland volume increased by 27% as compared with the NPD; this is similar to the parathyroid gland volume increase reported by Hernández et al. [9].

In conclusion, our data show that in normal rats, an HPD directly stimulates the parathyroid cell cycle independently of serum calcium and calcitriol.

Acknowledgements. The present study was supported by a grant from Consejería de Salud, Junta de Andalucía (JA 96/152), the Spanish Government (PM95-0184, FIS 95/235) and the Baxter Extramural Grant Program (1996). A. C. is being supported by a grant from the Spanish Ministry of Health (Beca de Perfeccionamiento, I. S. Carlos III, FIS Program); A. H. is being supported by a grant from the Spanish Ministry of Education (FPI Program) and Y. A. is sponsored by Fundacion Hospital Reina Sofía-CajaSur.

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