Effect of Propranolol on Phosphate Reabsorption by Superficial Nephron Segments in Response To Parathyroid Hormone in Phosphate-Deprived Rats

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ABSTRACT
Phosphate deprivation causes a resistance to the phosphaturic effect of parathyroid hormone. The decreased phosphaturic response to parathyroid hormone in rats fed a low phosphate diet for 1 day can be restored by propranolol infusion. Free-flow micropuncture studies were performed to localize the nephron site of restoration of the phosphaturic effect of parathyroid hormone by propranolol in rats deprived of phosphate for one day. In animals fed low phosphate diet and in the presence of parathyroid hormone, propranolol infusion did not change phosphate delivery to the late proximal tubule; however, fractional delivery of phosphate to the early distal tubule was significantly increased from 18.3 ± 2.9 to 32.2 ± 4.1%. In rats fed a normal phosphate diet, propranolol infusion did not change phosphate delivery along the nephron. We conclude that the restoration of the phosphaturic effect of parathyroid hormone by propranolol infusion in rats deprived of phosphate for 1 day is primarily due to decreased reabsorption of phosphate by superficial loop segments, most likely the pars recta segment of the proximal tubule.

Key Words: Micropuncture, parathyroid hormone, propranolol, low phosphate diet, phosphate reabsorption

Rats fed a low phosphate diet (LPD) have a decreased phosphaturic response to parathyroid hormone (PTH). The phosphaturic response to PTH can be restored by propranolol infusion in rats fed a low phosphate diet for 1 day, but not when they are subjected to longer periods of phosphate deprivation (1).

Although phosphate is reabsorbed in both the convoluted and the straight segments of the proximal tubule (2,3), the resistance to the inhibitory effect of PTH on phosphate reabsorption in rats fed a low phosphate diet for a short period of time was localized primarily between the late proximal and early distal tubules (4,5). Therefore, micropuncture experiments were performed to determine the nephron site of the restoration of the phosphaturic response to PTH by propranolol infusion in rats deprived of phosphate for 1 day.

METHODS
Experiments were performed on male Sprague-Dawley rats weighing 200–260 g. Animals were fed for 1 day on either LPD (0.07% phosphate, ICN Biomedicals Inc., Cleveland, OH) or normal phosphate diet (NPD, 0.7% phosphate). The NPD was prepared by supplementing the standard LPD with sodium and potassium phosphate. The ratio of monobasic to dibasic salts was 1:4, giving a final concentration of 0.7% of phosphate. For the animals fed the LPD, sodium and potassium were supplemented by NaCl and KCl to the same concentrations as the NPD.

The animals were housed in metabolic cages. The amount of diet consumed and urinary phosphate excretion were recorded daily. Rats were anesthetized by intraperitoneal injection of inactin (Byk Gulden, Konstanz, West Germany, 100 mg/kg b.w.) and were placed on a heated table. Body temperature was maintained between 36° and 38°C. A thyroparathyroidectomy (TPTX) by heat cautery and tracheostomy were performed. Catheters were inserted into a carotid artery for blood sampling and pressure monitoring, and into two jugular veins for infusions. After TPTX, a 2-h recovery period was allowed to establish a steady state. A 6% insulin in saline infusion was started at the beginning of the recovery period at a rate of 1.6 ml/h, and continued until the end of the experiment. The rats were also infused with 6.25% albumin in isotonic saline at a rate of 1% b.w./h for 1 hr. This infusion was then replaced by isotonic saline at a rate of 3% b.w./h. After 1 hr this infusion was decreased to equal the urine flow rate
Group 1 (N = 9): Low Phosphate Diet + PTH

Rats were fed with the LPD for 1 day before the experiment. Two hours after TPTX, 30-min control clearance samples were collected, and free-flow micropuncture samples were taken from the superficial late proximal and early distal tubules. Synthetic bovine PTH (1-34, Sigma Chemical Co., St. Louis, MO) was infused in a priming dose of 33 U/kg followed by continuous infusion of 1 U/kg/min. The experimental clearance period and micropuncture samples were collected 1 h after the hormone infusion was initiated.

Group 2 (N = 6): Low Phosphate Diet + Propranolol + PTH

The protocol was identical to that in group 1, except that 1 h after TPTX, propranolol (Inderal, Ayerst Laboratories, Inc., New York, NY) in isotonic saline was infused at 20 µg/kg/min until the end of the experiment.

Group 3 (N = 4): Low Phosphate Diet + Propranolol

The protocol was identical to that in group 2, except that PTH was not infused.

Group 4 (N = 3): Low Phosphate Diet (Time Control)

The protocol was identical to that in group 1, except that PTH was not infused.

Group 5 (N = 7): Normal Phosphate Diet + PTH

Animals were fed with the described NPD for 1 day before the experiment. The protocol was otherwise identical to that for group 1.

Group 6 (N = 7): Normal Phosphate Diet + Propranolol + PTH

The protocol was identical to that in group 5, except that propranolol was infused as in group 2.

Group 7 (N = 5): Normal Phosphate Diet + Propranolol

The protocol was identical to that in group 6, except that PTH was not infused.

Group 8 (N = 3): Normal Phosphate Diet (Time Control)

The protocol was identical to that in group 5, except that PTH was not infused.

Inulin concentrations in tubular fluid and plasma were determined by the microfluorimetric method of Vurek and Pegram (7). The concentration of inulin in urine was measured by the anthrone method (8). The volume of the tubule fluid was measured with a 1-µl constant bore capillary. Phosphate concentrations in plasma, urine, and tubule fluid were determined according to the method of Chen (9). The fractional delivery of phosphate was calculated from the tubule fluid to plasma phosphate and inulin ratios obtained from the same samples of tubule fluid. An aliquot of urine was frozen for subsequent determination of cAMP using a radiolimmunoassay kit (Biomedical Technologies, Inc., Stoughton, MA).

All values are presented as means ± SE. Comparisons were made using the t test for paired and unpaired data as appropriate. Significance was designated as P < 0.05.

RESULTS

The clearance data of animals fed a normal or low phosphate diet are summarized in Table 1. In rats fed the low phosphate diet, PTH infusion increased the fractional excretion of phosphate (FE\textsubscript{p}) from 0.4 ± 0.1 to 18.2 ± 2.5%, but when animals were pretreated with propranolol, PTH increased FE\textsubscript{p} to 30.4 ± 2.8%. In animals fed a normal phosphate diet, PTH administration significantly increased FE\textsubscript{p}, from 4.9 ± 1.9 to 39.0 ± 2.8%. Propranolol infusion did not change the phosphaturic response to PTH in these animals. Plasma phosphate concentration in the absence of PTH in rats fed a normal phosphate diet was significantly higher than after PTH infusion and higher than in phosphate deprived animals. Glomerular filtration rate was not altered by either propranolol or PTH. Blood pressure in rats fed a low phosphate diet was decreased after PTH infusion as compared to the control period, but it remained within the physiologic range. PTH infusion resulted in an increase in urinary cAMP excretion, but no differences were found between rats fed the low and normal phosphate diets both in the presence and absence of propranolol infusion (Table 1).

Tables 2 and 3 summarize the micropuncture data and single nephron proximal fluid-to-plasma inulin concentration ratio for the late proximal and early distal tubules were not signifi-
TABLE 1. Effect of PTH in TPTX rats fed low or normal phosphate diets in the presence and absence of propranolol*  

<table>
<thead>
<tr>
<th></th>
<th>MAP* (mmHg)</th>
<th>PN (mmol/L)</th>
<th>GFR (ml/min)</th>
<th>FEp (%)</th>
<th>cAMP (pmoles/mL GFR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-PRO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+PRO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>126 ± 4</td>
<td>126 ± 4</td>
<td>2.82 ± 0.12</td>
<td>3.03 ± 0.17</td>
<td>1.11 ± 0.05</td>
</tr>
<tr>
<td>PTH</td>
<td>115 ± 2*</td>
<td>115 ± 3*</td>
<td>2.83 ± 0.11</td>
<td>2.91 ± 0.18</td>
<td>1.14 ± 0.07</td>
</tr>
<tr>
<td>NPD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>130 ± 4</td>
<td>125 ± 3</td>
<td>3.54 ± 0.08</td>
<td>3.59 ± 0.10</td>
<td>1.00 ± 0.08</td>
</tr>
<tr>
<td>PTH</td>
<td>122 ± 4</td>
<td>125 ± 3</td>
<td>3.05 ± 0.11*</td>
<td>3.02 ± 0.13c</td>
<td>1.00 ± 0.11</td>
</tr>
</tbody>
</table>

* Values are means ± SE.

TABLE 2. Effect of PTH on phosphate delivery to the late proximal and early distal tubules in TPTX rats fed low phosphate diet in the presence and absence of propranolol*  

<table>
<thead>
<tr>
<th></th>
<th>SNGFR* (nl/min)</th>
<th>TF/Pn</th>
<th>TF/Pn</th>
<th>FDn (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-PRO</td>
<td>+PRO</td>
<td>-PRO</td>
<td>+PRO</td>
</tr>
<tr>
<td>Late proximal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>42.1 ± 2.4</td>
<td>42.2 ± 3.2</td>
<td>2.27 ± 0.11</td>
<td>2.32 ± 0.18</td>
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<tr>
<td>PTH</td>
<td>44.1 ± 4.4</td>
<td>41.6 ± 3.9</td>
<td>1.92 ± 0.18</td>
<td>1.98 ± 0.20</td>
</tr>
<tr>
<td>Early distal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>44.6 ± 4.5</td>
<td>46.4 ± 5.9</td>
<td>4.06 ± 0.40</td>
<td>4.45 ± 0.70</td>
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<tr>
<td>PTH</td>
<td>48.7 ± 4.8</td>
<td>48.7 ± 3.5</td>
<td>4.67 ± 0.65</td>
<td>4.07 ± 0.50</td>
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</tbody>
</table>

* Values are means ± SE.

TABLE 3. Effect of PTH on phosphate delivery to the late proximal and early distal tubule in TPTX rats fed normal phosphate diet in the presence and absence of propranolol*  

<table>
<thead>
<tr>
<th></th>
<th>SNGFR* (nl/min)</th>
<th>TF/Pn</th>
<th>TF/Pn</th>
<th>FDn (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-PRO</td>
<td>+PRO</td>
<td>-PRO</td>
<td>+PRO</td>
</tr>
<tr>
<td>Late proximal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>45.5 ± 1.8</td>
<td>48.2 ± 2.9</td>
<td>1.93 ± 0.19</td>
<td>2.05 ± 0.16</td>
</tr>
<tr>
<td>PTH</td>
<td>45.9 ± 2.4</td>
<td>47.0 ± 4.9</td>
<td>1.96 ± 0.17</td>
<td>1.95 ± 0.14</td>
</tr>
<tr>
<td>Early distal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>46.9 ± 4.6</td>
<td>50.1 ± 5.5</td>
<td>4.15 ± 0.45</td>
<td>4.34 ± 0.46</td>
</tr>
<tr>
<td>PTH</td>
<td>44.2 ± 8.7</td>
<td>48.9 ± 4.1</td>
<td>4.13 ± 0.54</td>
<td>3.54 ± 0.28</td>
</tr>
</tbody>
</table>

* Values are means ± SE.

See Table 2 for definitions of terms.

Significant difference compared with control period, group /test.
cantly changed. In phosphate-deprived rats, PTH administration significantly increased the tubular fluid-to-plasma phosphate concentration ratio (TF/FP) and fractional delivery of phosphate (FDp) at the late proximal tubule. Propranolol infusion did not influence this response to PTH (Table 2). In contrast, propranolol significantly increased the delivery of phosphate to the early distal tubule in the presence of PTH from 18.3 ± 2.9% to 32.2 ± 4.1%.

In the time control experiments for animals fed a low phosphate diet, no changes were observed between control and experimental periods in FDp at the late proximal and early distal tubules (11.4 ± 5.5 vs. 4.6 ± 0.4% and 2.3 ± 0.5 vs. 4.2 ± 1.9%, respectively). In the absence of PTH, propranolol infusion did not change phosphate delivery during the control and experimental periods to the late proximal and early distal tubules (10.8 ± 7.2% vs. 4.13 ± 0.74% and 1.34 ± 0.33 vs. 2.36 ± 0.89%, respectively). Therefore, in rats fed low phosphate diet, propranolol infusion potentiated the decrease of phosphate reabsorption in response to PTH only in nephron segments between the late proximal and early distal sites of collection.

The effect of PTH in rats fed a normal phosphate diet on phosphate delivery to the late proximal and early distal tubules in the absence and presence of propranolol is shown in Table 3. PTH infusion markedly increased FDp at the late proximal tubule in normal rats. The presence of propranolol did not change FDp in response to PH at the late proximal and early distal tubules in normal rats (Table 3).

In the time control group of rats fed a normal phosphate diet, no differences were observed in FDp between the control and experimental periods at the late proximal and early distal tubules (47.5 ± 12.0 vs. 42.4 ± 1.0% and 19.8 ± 5.3 vs. 27.2 ± 6.2%, respectively). In the absence of PTH, propranolol itself did not influence phosphate delivery to the late proximal and early distal tubules (48.8 ± 6.5 vs. 42.6 ± 5.5% and 27.7 ± 2.8 vs. 27.1 ± 1.4%, respectively). Thus, these data indicate that in rats fed normal phosphate diet, propranolol does not influence phosphate reabsorption.

DISCUSSION

In rats deprived of phosphate for 1 day and infused with propranolol, PTH infusion increased FDp at the early distal tubule puncture site of superficial nephrons to levels similar to those in rats fed a normal phosphate diet. Thus, β-blockade by propranolol restores the phosphaturic effect of PTH primarily by decreasing reabsorption of phosphate by superficial loop segments, most likely the pars recta segment of the proximal tubule (Figure 1). We favor the interpretation that the pars recta segment is the primary site of altered phosphate reabsorption because no significant reabsorption of phosphate has been demonstrated in other segments of the loop of Henle (10,11). However, since the distal tubule epithelium may be included between the late proximal and early distal puncture sites, the results do not exclude a contribution by the distal tubules. This is an important consideration because both β-receptors and phosphate reabsorption have been demonstrated in the distal tubule (12).

Plasma phosphate concentrations and the filtered load of phosphate were not affected by propranolol. In rats fed normal phosphate diet, plasma phosphate concentration and consequently filtered load of phosphate were significantly decreased after PTH administration. Propranolol infusion had no additional effect (Table 1). These data suggest that factors other than plasma phosphate concentration are responsible for the restoration of the phosphaturic effect of PTH by propranolol infusion in phosphate-deprived rats.

The present study confirms the widely accepted finding that the phosphaturic response to PTH of animals fed a low phosphate diet is decreased as compared with the control rats fed a normal diet. In addition, the present study is consistent with the recent findings that propranolol restores the phosphaturic effect of PTH in rats fed a low phosphate diet for 1 day and does not influence phosphate excretion in normal rats (Table 1). Propranolol infusion by itself does not induce a phosphaturia in rats fed low or normal phosphate diets. This indicates that the combination of both propranolol and PTH is necessary to inhibit the reabsorption of phosphate in rats deprived of phosphate.
In the absence of PTH, there was no inhibition of phosphate reabsorption by propranolol at any of the nephron sites that were sampled in rats fed low or normal phosphate diets. Thus, propranolol itself does not inhibit phosphate reabsorption in either the proximal convoluted or the proximal straight tubule.

The finding that propranolol restores the phosphaturic effect of PTH primarily by inhibiting phosphate reabsorption in the pars recta segment of the proximal tubule is consistent with several other models of phosphate conservation. Different responses of phosphate reabsorption between proximal convoluted and proximal straight segments following PTH administration have been demonstrated in respiratory alkalosis (13), low phosphate diet (1,3,5), and in response to calcitonin (6) or nicotinamide administration (2). In all of these studies, the final excretion of phosphate in the urine paralleled the change in phosphate reabsorption by the pars recta segment of the proximal tubule and suggests that this segment may critically influence phosphate excretion in the final urine.

Recent studies indicate that the β-adrenergic system may play a role in the regulation of phosphate balance. Stimulation of β-receptors may be the coupling event between respiratory alkalosis or low phosphate diet and desensitization to PTH (1,14). The present study indicates that β-blockade may be necessary to restore the chain of events between PTH binding and inhibition of phosphate reabsorption, which is dissociated during low phosphate diet. This defect is probably between cAMP formation and phosphate reabsorption, because a normal increase of cAMP was observed in low phosphate diet rats after PTH (15). Moreover, propranolol infusion had no effect on urinary cAMP excretion in rats fed either low or normal phosphate diet (Table 1). These observations suggest that the restoration of the phosphaturic effect of PTH by propranolol through increased renal cAMP production is unlikely. Thus, propranolol probably modulates the PTH response at a step independent from cAMP formation and suggests the existence of β-receptors that are not necessarily associated with adenylate cyclase. However, other effects of propranolol, such as those on membrane fluidity, cannot be excluded.

In summary, the restoration of the phosphaturic effect of PTH by propranolol infusion in rats deprived of phosphate for 1 day is primarily due to decreased reabsorption of phosphate by superficial loop segments, most likely the pars recta.

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REFERENCES