Abnormal calcaemic response to PTH in the uraemic rat without secondary hyperparathyroidism


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Abstract

Background. Skeletal resistance to the calcaemic action of parathyroid hormone (PTH) is an important pathogenic factor in the development of secondary hyperparathyroidism. Since parathyroidectomy normalizes the calcaemic response to PTH in uraemic animals, the increase in PTH levels has been advanced as a cause of skeletal resistance to the calcaemic action of PTH. This study was designed to evaluate in uraemic rats the effect of normal PTH levels on the calcaemic response to PTH.

Methods. To maintain normal PTH levels, rats were parathyroidectomized (PTX) and rat 1–34 PTH was infused at a rate of 0.022 ug/100 g per hour via a subcutaneously implanted miniosmotic pump; this rate of infusion was considered to be the normal PTH replacement dose since it normalized serum calcium and phosphorus in PTX rats with normal renal function. Two separate studies were performed. In the first study, rats were maintained on a moderate-phosphorus (0.6%) diet and rats were divided into four groups: (I) normal; (II) uraemic; (III) PTX with normal PTH replacement; and (IV) uraemic with PTX and normal PTH replacement. In a second study, the groups were the same except that a high-phosphorus (1.2%) diet was given to increase the magnitude of hyperparathyroidism in rats with intact parathyroid glands; an additional group (V) identical to group IV except that rats received daily calcitriol was included. After 14 days, rats received a 48-h infusion of high-dose rat 1–34 PTH (0.11 ug/100 g per hour) to evaluate the calcaemic response to PTH.

Results. The calcaemic response to PTH was similar in normal rats and PTX rats with PTH replacement on both a moderate and high-phosphorus diet. In uraemic rats, the calcaemic response to PTH was decreased and the maintenance of normal PTH levels by PTH replacement did not correct the decreased calcaemic response to PTH; moreover, calcitriol supplementation did not improve the calcaemic response to PTH. Finally, hypocalcaemia was observed in uraemic rats with PTH replacement and was more profound than in rats on a high-phosphorus diet.

Conclusions. This study demonstrates that the maintenance of a normal PTH level in uraemic rats did not correct the impaired calcaemic response to PTH, suggesting that factors intrinsic to uraemia, independent of phosphorus, calcitriol, and PTH participate in the decreased calcaemic response to PTH in uraemia.

Key words: phosphorus; PTH; secondary hyperparathyroidism; skeletal resistance; uraemia

Introduction

Skeletal resistance to the calcaemic action of parathyroid hormone (PTH) is an important pathogenic factor in the development of secondary hyperparathyroidism [1–5]. In renal failure, serum calcium is often decreased despite markedly increased PTH levels. This is in part due to a decreased bone response to the calcaemic action of PTH. Several factors have been reported to contribute to the decreased calcaemic response to PTH in uraemia. These include phosphorus retention [6–8], a calcitriol deficiency [9,10], high-PTH-induced downregulation of the PTH receptor [10,11], and other factors intrinsic to uraemia [12].

Studies in uraemic animals with secondary hyperparathyroidism have shown that the elimination of circulating PTH by parathyroidectomy (PTX) corrects the impaired calcaemic response to PTH [10,11]. These results have been interpreted to suggest that the high PTH levels downregulate the bone PTH receptor. Moreover recent reports have shown that the expression of the PTH receptor mRNA is decreased in kidney, liver, heart, and bone of uraemic rats with secondary hyperparathyroidism [13–16]; however it remains to be evaluated whether the expression of the PTH receptor protein is also decreased in uraemia. Recently it has been demonstrated that in vitamin-D-deficient rats [17], there is a dissociation between gene message, its final product, and the transduction system.
While it may be attractive to attribute the decreased calcaemic response to PTH in uraemia to the high PTH levels, Bover et al. [12] have recently reported that the prevention of secondary hyperparathyroidism in uraemic rats by dietary phosphorus restriction did not normalize the calcaemic response to PTH. Moreover these investigators observed that the calcaemic response to PTH was decreased in uraemic PTX rats as compared with PTX rats with normal renal function. Another finding that has questioned the role of high PTH levels as the cause of downregulation of the PTH receptor is the recent report by Üreña et al. [18] in which it was observed that in uraemic rats, PTH receptor mRNA was decreased despite PTX. Thus these latter results would suggest that the mechanism by which PTX restores the calcaemic response to PTH to normal in uraemia may not be due to downregulation of the PTH receptor.

The aim of this study was to evaluate the independent roles of sustained elevation of PTH levels and uraemia on the calcaemic response to PTH. To accomplish these goals, the calcaemic response to PTH was evaluated in both normal and uraemic rats with either intact parathyroid glands or PTX receiving a replacement dose of PTH.

**Subjects and methods**

The study was performed in male Wistar rats weighing 130–150 g. Rats were placed in individual cages and underwent either sham operation or two-stage 5/6 nephrectomy; the latter was accomplished by ligation of two of the three main arteries of the left kidney followed one week later by right nephrectomy. Selective PTX was performed in one-half of the sham-operated and one-half of the 5/6 nephrectomized rats. This surgical intervention was done at the time of either the sham-operation or the arterial ligation; the PTX was considered successful only if the serum calcium was lower than 7.0 mg/dl 3 days after PTX. Before the initiation of the study period all rats received a 0.6% calcium, 0.6% phosphorus diet.

The dose of PTH required for hormonal replacement was assessed in preliminary experiments in PTX rats with normal renal function. Parathryoidectomized rats received a continuous administration of rat 1-34 PTH (Bachem, Torrance, California, USA) using a subcutaneously implanted miniosmotic Alzet pump (model 2001) at a constant rate of 0.11 μg/100 g per hour. During the PTH infusion, rats were fed a calcium-free and low-phosphorus (0.16%) diet to prevent intestinal absorption of calcium and avoid the inhibitory effect of phosphorus on the bone release of calcium [8]. Blood for measurement of serum calcium, phosphorus and creatinine was obtained immediately before and after the PTH infusion. Serum PTH levels were determined in rats with intact parathyroid glands immediately before the 48-h PTH infusion.

**Study 1. Calcaemic response to PTH in uraemic rats fed a moderate-phosphorus diet**

The following four groups were evaluated: (1) group I (sham operated), sham operated rats with intact parathyroid glands; (2) group II (Nx), 5/6 nephrectomized rats with intact parathyroid glands; (3) group III (sham operated with continuous PTH infusion), PTX rats with normal renal function and PTH replacement; and (4) group IV (Nx with continuous PTH infusion), 5/6 nephrectomized PTX rats with PTH replacement. After 14 days on the moderate-phosphorus diet, the calcaemic response to PTH was evaluated in the four groups. The method used to assess the calcaemic response to PTH has been described in our previous studies [8,10,12]. Briefly, after a 12-h fast, rat 1-34 PTH was administered via a subcutaneously implanted Alzet pump (model 2001) at a constant rate of 0.11 μg/100 g per hour for 48 h. During the PTH infusion, rats were fed a calcium-free and low-phosphorus (0.16%) diet to prevent intestinal absorption of calcium and avoid the inhibitory effect of phosphorus on the bone release of calcium [8]. Blood for measurement of serum calcium, phosphorus and creatinine was obtained immediately before and after the PTH infusion. Serum PTH levels were determined in rats with intact parathyroid glands immediately before the 48-h PTH infusion.

**Study 2. Calcaemic response to PTH in uraemic rats fed a high-phosphorus diet**

In this second study the groups were identical to those described in study 1 except that rats received a high-phosphorus diet to enhance the development of secondary hyperparathyroidism. The following groups were included: (1) group I (sham operated); (2) group II (Nx); (3) group III (sham operated with continuous PTH infusion); and (4) group IV (Nx with continuous PTH infusion). Since in the last group the serum calcitriol level was lower than in the Nx group (52±7 versus 74±5 pg/ml, P<0.05), a fifth group (Nx with continuous PTH infusion plus calcitriol) was added to evaluate whether a calcitriol deficiency could be responsible for some of the difference in the calcaemic response to PTH. In this group a daily dose of calcitriol (20 pmol) was administered subcutaneously throughout the entire experimental period including the last 48 h in which the calcaemic response to PTH was evaluated. This dose of calcitriol has been shown to increase calcitriol levels and decrease parathyroid cell proliferation without producing hypercalcaemia in uraemic rats [20]. During the last 48 h of study the rats underwent PTH infusion to assess the calcaemic response as described in study 1. In addition, serum calcitriol levels were determined before the 48-h PTH infusion in a subgroup of rats from the Nx (n=7) and Nx with continuous PTH infusion (n=7) groups.

Serum calcium, phosphorus, and creatinine were measured using standard automated laboratory techniques. Intact PTH was measured with an IRMA assay specific for rats (Nichols, San Juan Capistrano, California, USA); the intra- and interassay coefficients of variation were 5.8% and 9% respectively. With this assay we were unable to quantify serum PTH in rats receiving an exogenous PTH infusion. Serum calcitriol
**STUDY 1. Moderate-phosphorus diet**

- **Group I:** Sham
- **Group II:** 5/6Nx
- **Group III:** Sham + PTX
- **Group IV:** 5/6Nx + PTX

**Moderate-phosphorus diet (P, 0.6%; Ca, 0.6%)**

**Day 0, 14, 16**

**PTH inf (0.022 μg/100 g/h)**

**STUDY 2. High-phosphorus diet**

- **Group I:** Sham
- **Group II:** 5/6Nx
- **Group III:** Sham + PTX
- **Group IV:** 5/6Nx + PTX
- **Group V:** 5/6Nx + PTX + calcitriol (20 pmol/day)

**High-phosphorus diet (P, 1.2%; Ca, 0.6%)**

**Day 0, 14, 16**

**PTH inf (0.11 μg/100 g/h)**

was measured with a radioreceptor assay (Nichols, San Juan Capistrano, California, USA); the intra- and interassay coefficients of variation were 7.2% and 10.4% respectively.

**Statistical analysis**

Differences between the means of groups on the same phosphorus diet were evaluated by one-way ANOVA followed by a multiple comparison test (Duncan). A *P* value <0.05 was considered significant. Results are expressed as the mean ± SE.

**Results**

**Calcemic response to PTH in uraemic rats fed a moderate-phosphorus diet**

After 14 days on a moderate-phosphorus diet, the serum calcium was similar in rats with normal renal function, Nx rats with intact parathyroid glands, and rats with normal renal function with PTH replacement; in contrast, serum calcium was significantly decreased (*P* <0.01) in the Nx-PTH replaced group (Figure 2A, Table 1). Serum PTH levels were approximately sixfold greater (*P* <0.05) in the Nx-PTH replaced group than the other four groups (Table 2, Figure 3). After the 48-h PTH infusion the serum calcium and the delta calcium were greater (*P* <0.05) in the two groups with normal renal function than the three Nx groups; moreover, among the Nx groups, the delta calcium was less (*P* <0.05) in the parathyroid intact than the two groups with PTH replacement (Table 2, Figure 3). The PTH level in Nx rats with intact parathyroid glands was more than sixfold greater than in the group with normal renal function and intact parathyroid glands (51.3 ± 8.7 versus 7.3 ± 1.3 pmol, *P* <0.001). Before the PTH infusion, the serum phosphorus was greater in rats with normal renal function and PTH replacement than in normal renal function with intact parathyroid glands (3.02 ± 0.11 versus 2.23 ± 0.09 mmol, *P* <0.001), but less than in the three Nx groups (Table 2). In all replaced groups for the same renal function; however, there was an approximate sixfold difference in PTH levels between the two Nx groups. Before the PTH infusion test, the serum phosphorus level was elevated in the Nx-PTH replaced group (*P* <0.05); after the PTH infusion the serum phosphorus decreased to similar levels in all groups (Table 1).

**The calcemic response to PTH in uraemic rats fed a high-phosphorus diet**

After 14 days on a high-phosphorus diet, the serum calcium was greater (*P* <0.05) in the group of rats with normal renal function and intact parathyroid glands than in the other four groups and lower (*P* <0.05) in the Nx-PTH replaced group than the other four groups (Table 2, Figure 3). After the 48-h PTH infusion the serum calcium and the delta calcium were greater (*P* <0.05) in the two groups with normal renal function than the three Nx groups; moreover, among the Nx groups, the delta calcium was less (*P* <0.05) in the parathyroid intact than the two groups with PTH replacement (Table 2, Figure 3). The PTH level in Nx rats with intact parathyroid glands was more than sixfold greater than in the group with normal renal function and intact parathyroid glands (51.3 ± 8.7 versus 7.3 ± 1.3 pmol, *P* <0.001). Before the PTH infusion, the serum phosphorus was greater in rats with normal renal function and PTH replacement than in normal renal function with intact parathyroid glands (3.02 ± 0.11 versus 2.23 ± 0.09 mmol, *P* <0.001), but less than in the three Nx groups (Table 2). In all
Abnormal calcaemic response to PTH in uraemia

**Table 1. Moderate-phosphorus diet**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sham op. (n = 12)</th>
<th>Nx (n = 7)</th>
<th>Sham op. + continuous PTH infusion (n = 10)</th>
<th>Nx + continuous PTH infusion (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. creatinine (μmol)</td>
<td>26 ± 1.7</td>
<td>62 ± 2.6</td>
<td>28 ± 2.8</td>
<td>59 ± 3.1</td>
</tr>
<tr>
<td>S. calcium before (mmol)</td>
<td>2.50 ± 0.02</td>
<td>2.52 ± 0.05</td>
<td>2.45 ± 0.02</td>
<td>2.17 ± 0.05</td>
</tr>
<tr>
<td>S. calcium after</td>
<td>4.35 ± 0.10</td>
<td>3.47 ± 0.12</td>
<td>4.10 ± 0.11</td>
<td>3.12 ± 0.09</td>
</tr>
<tr>
<td>S. phosphorus before (mmol)</td>
<td>2.40 ± 0.06</td>
<td>2.40 ± 0.10</td>
<td>2.53 ± 0.10</td>
<td>2.86 ± 0.13</td>
</tr>
<tr>
<td>S. phosphorus after</td>
<td>1.91 ± 0.16</td>
<td>1.85 ± 0.19</td>
<td>1.90 ± 0.13</td>
<td>2.13 ± 0.13</td>
</tr>
</tbody>
</table>

*p < 0.05 versus a sham operated, b Nx, c sham operated plus continuous PTH infusion.*

that normal PTH levels in uraemic rats did not correct the calcaemic response to PTH. Moreover normal PTH levels were unable to maintain a normal serum concentration of calcium and phosphorus in uraemic rats on a moderate-phosphorus diet and both non-uraemic and uraemic rats on a high-phosphorus diet. Our results would suggest that in uraemia the decreased calcaemic response to PTH is not due to a downregulation of PTH receptor by high PTH levels. Therefore additional factors intrinsic to uraemia likely contribute to the decreased calcaemic response to PTH.

In rats on a moderate-phosphorus diet, our PTH replacement dose (0.022 μg/100 g per hour) resulted in a normal serum calcium and phosphorus level in rats with normal renal function. Lower PTH doses resulted in hypocalcaemia and higher PTH doses in hypercalcemia. Thus, even though the PTH level could not be measured with a conventional assay, the PTH dose used as a replacement dose in the PTX groups would appear to be appropriate for the maintenance of calcium and phosphorus homeostasis in the normal rat. Since in PTX rats the PTH replacement dose was fixed, this would prevent the development of secondary hyperparathyroidism during uraemia irrespective of the demands of the system. Thus, high PTH levels inducing downregulation of the PTH receptor should be prevented.

The consequences of preventing an increase in PTH levels when demands are placed on the system for calcium and phosphorus homeostasis are demonstrated by the serum calcium and phosphorus concentration after 14 days of study diet. In rats with PTH replacement and normal renal function, a normal serum calcium and phosphorus concentration was maintained when rats received a moderate-phosphorus diet, but hypocalcaemia and hyperphosphataemia were observed in rats on a high-phosphorus diet. It must be assumed that the inability to increase PTH levels during phosphorus loading resulted in hypocalcaemia and hyperphosphataemia even when renal function was normal; this assumption is based on the fact that normal rats with intact parathyroid glands on a high-phosphorus diet were able to maintain a normal serum calcium and phosphorus and this result was presumably due to the threefold increase in PTH levels. In uraemic rats, the results were more dramatic. In the uraemic rats on a moderate phosphorus diet, a modest

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**Fig. 2.** Serum calcium concentration in rats before and after a 48-h PTH infusion test (0.11 μg/100 g per hour) (A), and the corresponding delta calcium (B) in different groups of rats fed a moderate-phosphorus diet (P = 0.6%). Nx, 5/6 nephrectomized rats. Groups with continuous PTH infusion are parathyroidectomized rats with exogenous rat PTH replacement (0.022 μg/100 g per hour). Results are mean ± SE; *P < 0.05 versus (a) sham operated, (b) Nx, (c) sham operated with continuous PTH infusion.
Table 2. High-phosphorus diet

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sham op. (n = 14)</th>
<th>Nx (n = 7)</th>
<th>Sham op. + continuous PTH infusion (n = 11)</th>
<th>Nx + continuous PTH infusion (n = 7)</th>
<th>Nx + continuous PTH infusion + Calcitriol (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (µmol)</td>
<td>28 ± 1.4</td>
<td>72 ± 3.8a</td>
<td>30 ± 1.8b</td>
<td>63 ± 3.0bc</td>
<td>70 ± 4.5c</td>
</tr>
<tr>
<td>Calcium before (mmol)</td>
<td>2.52 ± 0.03</td>
<td>2.07 ± 0.10a</td>
<td>2.25 ± 0.06a</td>
<td>1.62 ± 0.07bc</td>
<td>2.30 ± 0.11bc</td>
</tr>
<tr>
<td>Calcium after</td>
<td>4.13 ± 0.06</td>
<td>2.80 ± 0.12a</td>
<td>3.92 ± 0.11b</td>
<td>2.87 ± 0.12bc</td>
<td>3.55 ± 0.10bc</td>
</tr>
<tr>
<td>Phosphorus before (mmol)</td>
<td>2.23 ± 0.09</td>
<td>4.16 ± 0.36a</td>
<td>3.02 ± 0.11ab</td>
<td>3.70 ± 0.23ab</td>
<td>3.93 ± 0.31ab</td>
</tr>
<tr>
<td>Phosphorus after</td>
<td>1.60 ± 0.12</td>
<td>1.53 ± 0.17</td>
<td>1.76 ± 0.13</td>
<td>1.73 ± 0.16</td>
<td>1.86 ± 0.17</td>
</tr>
</tbody>
</table>

P < 0.05 versus * sham operated, a Nx, b sham operated with continuous PTH infusion, c Nx with continuous PTH infusion.

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A decrease in serum calcium was observed in the PTH replacement group, but not in the group with intact parathyroid glands; in the latter group, an approximately sixfold increase in PTH was able to maintain a normal serum calcium. In the uraemic rats on a high-phosphorus diet, hypocalcaemia and hyperphosphataemia were observed in the parathyroid intact group despite an almost 20-fold increase in PTH levels; however, in uraemic rats with PTH replacement, the hypocalcaemia was considerably more profound. Thus these results demonstrate that a distinct failure to regulate serum calcium and phosphorus is present in uraemia when PTH levels are unable to adapt to the demands of the system [21].

Fig. 3. Serum calcium concentration in rats before and after a 48-h period PTH infusion test (0.11 µg/100 g per hour) (A), and the corresponding delta calcium (B) in different groups of rats fed a high-phosphorus diet (P = 1.2%). Nx, 5/6 nephrectomized rats. Groups with continuous PTH infusion are parathyroidectomized rats with exogenous rat PTH replacement (0.022 µg/100 g per hour). The Nx + calcitriol group received calcitriol (20 pmol/day) in addition to the continuous PTH infusion. Results are mean ± SE. P < 0.05 versus (a) sham operated, (b) Nx, (c) sham operated with continuous PTH infusion (d) Nx with continuous PTH infusion.

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In the first study in which rats were fed a moderate-phosphorus diet, uraemic rats had a decreased calcaemic response as compared with the non-uraemic rats and it was not corrected by normal PTH levels. This suggests that uraemia rather than high PTH was responsible for the decreased calcaemic response to PTH. It is also likely that the decreased calcaemic response to PTH in uraemic rats with PTH replacement was in part responsible for the observed hypocalcaemia. The cause of the decreased calcaemic response to PTH was probably not due to hyperphosphataemia, since the 48-h PTH infusion was performed with rats on a low-phosphorus diet and the post-infusion serum phosphorus level were similar in all groups. Thus uraemia per se may have been a contributing factor.

In the second study, rats were fed a high-phosphorus diet which enhanced the severity of secondary hyperparathyroidism as demonstrated by the fact that in uraemic rats on a high-phosphorus diet, PTH levels were more than threefold greater than uraemic rats on a moderate-phosphorus diet (51.3 ± 8.7 versus 15.4 ± 4.8 pmol). Similar to rats fed a moderate-phosphorus diet (study 1), the calcaemic response to
difficult to observe in the present study if in our calcaemic response to PTH was due to a PTH induced downregulation of bone PTH receptors in the uraemic rats with intact parathyroid glands and markedly increased PTH. However, a second explanation for this observation could be that the high degree of bone remodelling in parathyroid-intact uremic rats [22] could have affected the calcaemic response to PTH since Parfitt [23,24] has postulated that the homeostatic process which maintains the serum calcium concentration is dependent on the extent of quiescent bone surfaces.

Earlier studies in uraemic animals have demonstrated that PTX restored the calcaemic response to PTH to normal [10,11] and thus suggested that downregulation of PTH receptors was induced by high PTH levels. Although a decreased expression of PTH receptors have been shown to be present in uraemic animals [13–16] and could have been due to high PTH levels, Ureña et al. [18] have reported that a decreased expression of PTH receptor mRNA was observed even in PTX uraemic rats and thus the decreased expression of PTH receptor mRNA could not be caused by high PTH levels. While decreased expression of PTH receptor mRNA is one of the mechanisms of downregulation, in the present study the term downregulation refers to abnormal receptor function directly caused by continuous exposure of the receptor to high PTH levels. In the study by Bover et al. [12] in which hyperparathyroidism was prevented by a phosphorus-restricted diet, the calcaemic response to PTH was decreased in uraemic rats despite normal PTH levels and among PTX rats, the calcaemic response was lower in uraemics than normals. Thus, by demonstrating that the calcaemic response to PTH was decreased in uraemic rats despite normal PTH levels and among PTX rats, the calcaemic response was lower in uraemics than normals. This decrease in calcaemic response cannot be explained by the high PTH levels, and it would appear that downregulation of PTH receptor due to high PTH levels does not play a major role in the decreased calcaemic response to PTH in uraemia.

Some previous studies have reported that calcitriol supplementation improves the calcaemic response to PTH in uraemia [8,9], but this finding was not confirmed by others [10]. In the present study, calcitriol supplementation did not improve the calcaemic response to PTH. It is possible that calcitriol may increase the calcaemic response by decreasing bone remodelling [25–27]; however, this effect would be difficult to observe in the present study if in our nephrectomized PTX rats with PTH replacement receiving calcitriol, bone turnover was not increased due to the normal PTH level.

In conclusion, this study demonstrates that the main-tenance of normal PTH levels in uraemic rats did not correct the impaired calcaemic response to PTH. Moreover, our findings would indicate that uraemia, independent of PTH and calcitriol, resulted in a decreased calcaemic response to PTH. Finally, it is unlikely that high PTH levels are a critical factor in the decreased calcaemic response to PTH observed in uraemia.

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References


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