Regulation of intestinal vitamin D receptor expression in experimental uraemia: effects of parathyroidectomy and administration of PTH

András Szabó1,2, Axel Schmutz1, Siamak Pesian1, Heinrich Schmidt-Gayk1,*, Eberhard Ritz1 and Helmut Reichel1

1Department of Internal Medicine, Division of Nephrology, University of Heidelberg, Germany; and 21st Department of Pediatrics, Semmelweis University, Budapest, Hungary

Abstract In this study, the effects of PTH on binding of [3H]-1,25-dihydroxyvitamin D3 [1,25(OH)2D3] and on vitamin D receptor (VDR) mRNA concentration were assessed in intestinal mucosa of subtotally nephrectomized rats (Nx) and in intestinal mucosa of sham-operated rats with normal kidney function (Intact). Intestinal 1,25(OH)2D3 binding capacity of Intact remained unchanged (i) after parathyroidectomy (PTx), (ii) after administration of PTH for up to 6 days, and (iii) after PTx and subsequent administration of PTH (n = 4 experiments). In contrast, PTx of subtotally nephrectomized animals (Nx-PTx) decreased 1,25(OH)2D3 binding capacity from 757 ± 95 fmol/mg protein in Nx to 417 ± 42 in Nx-PTx (P < 0.01, n = 5). PTH administration had no effect on intestinal 1,25(OH)2D3 binding capacity in Nx. However, PTH administration to Nx-PTx resulted in re-elevation of 1,25(OH)2D3 binding capacity to a level (790 ± 113 fmol/mg protein) which was comparable to Nx. Ka-values remained unaltered under all experimental conditions. The intestinal VDR mRNA concentration (normalized to β-actin mRNA) was decreased, on average, by 23% in Nx-PTx (P < 0.05 versus Nx).

In further experiments, 1,25(OH)2D3 was administered to Nx-PTx. This resulted in upregulation of 1,25(OH)2D3 binding capacity as compared to vehicle-treated Nx-PTx (562 ± 90 fmol/mg protein versus 249 ± 32, P < 0.01). The latter results could indicate that PTH-mediated stimulation of residual renal 1,25(OH)2D3 production was involved in PTH-mediated up-regulation of intestinal 1,25(OH)2D3 binding capacity in Nx-PTx. To rule out this possibility, PTH was administered to totally nephrectomized and parathyroidectomized rats (TNx-PTx). Since PTH caused an approximately 80% increase (P < 0.05) in intestinal 1,25(OH)2D3 binding capacity under those experimental conditions a mediator role of 1,25(OH)2D3 could be excluded.

Functional significance of decreased intestinal 1,25(OH)2D3 binding capacity in Nx-PTx as compared to Nx was demonstrated by significantly lower 1,25(OH)2D3-mediated stimulation of intestinal 25(OH)D3-24-hydroxylase activity in Nx-PTx (209 ± 68 pmol/mg protein) than in Nx (385 ± 81, P < 0.01). The modulation of intestinal 1,25(OH)2D3 binding capacity was not correlated with changes in calcium, phosphate or 1,25(OH)2D3 serum concentrations under our experimental conditions. Taken together, intact parathyroid gland function was required to maintain adequate intestinal VDR expression in experimental uraemia (but not in normal animals). The mechanism of the modulation of intestinal VDR by PTH remains to be elucidated although an indirect effect of PTH on VDR expression in intestinal mucosa seems most likely.

Key words: calcium metabolism; vitamin D receptor; uraemia; parathyroid hormone; vitamin D

Abbreviations: 1,25(OH)2D3, 1α,25-dihydroxyvitamin D3; 25(OH)D3, 25-hydroxyvitamin D3; PTH, parathyroid hormone; VDR, vitamin D receptor; Nx, subtotal nephrectomy; TNx, total nephrectomy; PTx, parathyroidectomy.

Introduction

The involvement of PTH in the regulation of VDR expression was first suggested in 1988 by Pols et al. [1] in that PTH (1–34) elevated the 1,25(OH)2D3 binding capacity of osteoblasts approximately four-fold. This was preceded by a rise in intracellular cAMP and VDR mRNA concentrations [2]. In contrast to those results, PTH (1–34) caused downregulation of VDR mRNA and 1,25(OH)2D3 binding capacity in ROS osteosarcoma cells [3]. Experiments in vivo with intact rats failed to demonstrate an effect of the administration...
of PTH on intestinal 1,25(OH)$_2$D$_3$ binding capacity. However, administration of PTH blocked 1,25(OH)$_2$D$_3$-mediated upregulation of intestinal 1,25(OH)$_2$D$_3$ binding capacity [3]. This was compatible with the finding that an elevation of endogenous 1,25(OH)$_2$D$_3$ synthesis through hypocalcaemic diet (which produces secondary hyperparathyroidism) did not result in upregulation of intestinal 1,25(OH)$_2$D$_3$ binding capacity whereas exogenous administration of 1,25(OH)$_2$D$_3$ (which is not associated with secondary hyperparathyroidism) led to homologous upregulation of VDR [4]. Further support for an involvement of PTH in the regulation of VDR expression stems from additional studies in vitro [5–7] utilizing osteoblast-like and cartilage cells, and from a clinical study which reported a higher 1,25(OH)$_2$D$_3$ binding capacity in mononuclear cells from patients with primary hyperparathyroidism than in cells from normal subjects (8).

Abnormal expression and regulation of VDR in renal failure [9–13] may be of importance for pathogenesis of renal hyperparathyroidism. The aim of the current study was to assess the role of PTH in the regulation of intestinal 1,25(OH)$_2$D$_3$ binding capacity and VDR mRNA concentration in experimental renal failure.

Subjects and methods

Animals and surgical procedures

Male Sprague–Dawley rats (Ivanovas, Kisslegg, Germany), 7–8 weeks and 170–190 g, were housed in an environment with controlled light (12 h on/12 h off cycles), constant temperature (22°C) and humidity (70%). The animals had free access to deionized water and standard diet (400 U vitamin D$_3$/kg, 0.95% calcium, 0.75% phosphate (weight/ weight; Altromin, Lage, Germany). All surgical procedures were performed under Ketamine (Parke-Davis, Berlin, Germany) anaesthesia.

Subtotal Nephrectomy (Nx)

Animals were subjected to a two-step subtotal (5/6) nephrectomy (Nx) procedure. During the first surgery, one kidney was resected. A 2/3 nephrectomy of the second kidney was performed 48–96 h later. Control animals were sham-operated by decapsulation of kidneys (Intact).

Parathyroidectomy (PTx)

Parathyroid glands were removed by microsurgical technique during surgery for resection of the second kidney (in Nx). Sham-operated control animals were parathyroidectomized immediately before the beginning of treatment. The PTx was verified by reduced fasting serum calcium concentration (below 2.0 mmol/l) 2 days after PTx. Blood samples were collected by retro-orbital puncture. Control animals were sham-operated by neck dissection and surgical preparation of parathyroid glands.

Total nephrectomy (TNx)

For experiments in totally nephrectomized rats, animals were unilaterally nephrectomized. The second kidney was removed 6 days after the first operation; animals were parathyroidectomized at the same time (where indicated). In those animals, measurement of serum calcium after PTx was not possible.

Osmotic minipumps

Osmotic minipumps (Alzet 2001, Alza Research Inc., Palo Alto, USA) were implanted subcutaneously in the neck of the animals at the time-point of second nephrectomy. The function of minipumps was verified as described previously [14].

Experimental procedures

If not indicated otherwise, experimental groups included 6–10 animals. In each experiment, 2–3 separate vitamin D receptor measurements were carried out. Pooled tissue from 2 to 5 animals was utilized for each measurement. If not indicated otherwise, tissues were collected 6 days after second Nx or sham operation.

Effect of PTx on intestinal 1,25(OH)$_2$D$_3$ binding capacity

Both in Nx and Intact, vehicle-treated or PTH-treated parathyroidectomized animals were sacrificed 6 days after PTx.

Effect of PTH administration on intestinal 1,25(OH)$_2$D$_3$ binding capacity

PTH was administered continuously for 6 days. Rat PTH(1-34, Bachem, Heidelberg, Germany), dissolved in NaCl 0.9% and 2% L-cysteine (Serva), was infused by osmotic minipump at a dose of 100 ng/kg bodyweight/day. This dose was chosen since pilot experiments had shown that this amount of PTH did not cause hypercalcaemia. Experiments were terminated after the 6-day treatment period with PTH, and tissues were collected. In Nx, PTH-infusion was started at the time-point of second nephrectomy immediately after PTx. In Intact, PTH-infusion was started immediately after PTx. In one experiment (as indicated), 1 µg PTH was administered as a subcutaneous injection 24 h prior to the termination of the experiment (6 days after PTx). Control groups received the appropriate vehicle.

In Nx, three experiments were carried out which comprised Nx, Nx-PTx and Nx-PTx + PTH. In those experiments, VDR mRNA concentration was also measured.

Reversibility of the effect of PTx on intestinal 1,25(OH)$_2$D$_3$ binding capacity

Three groups of animals were formed: (i) Nx; (ii) Nx-PTx; (iii) Nx-PTx and infusion of PTH for 48 h by osmotic minipump (100 ng/kg bodyweight/day) starting 6 days after second Nx. Groups (i) and (ii) received vehicle by osmotic minipump. The experiment was completed 8 days after the last surgery.
Effect of 1,25(OH)₂D₃ on intestinal 1,25(OH)₂D₃ binding capacity in Nx-PTx

In additional experiments in Intact and Nx, parathyroidec-tomized animals received 1,25(OH)₂D₃ (100 pmol/kg bodyweight/day) by osmotic minipump for 6 days. Infusion of 1,25(OH)₂D₃ was started immediately after PTx. In one experiment (as indicated), the 1,25(OH)₂D₃ was given by intraperitoneal injection (100 pmol) 6 days after the second Nx. The experiment was terminated 12 h after injection of 1,25(OH)₂D₃. The 1,25(OH)₂D₃ (a generous gift of Dr Calcanis, Hoffman-LaRoche, Inc., Grenzach, Germany) had been stored at −20 °C in absolute ethanol and had been dissolved for experiments in propylene glycol. The control groups received the appropriate vehicle.

Effect of PTH on intestinal 1,25(OH)₂D₃ binding capacity in total nephrectomy (TNx) and PTx

Three groups of animals (n = 12) were studied: (i) TNx; (ii) TNx-PTx; (iii) TNx-PTx and injection of 1 μg rat PTH (1–34) i.p. immediately after second Nx and PTx. Experiments were completed 12 h after second nephrectomy. For 48 h prior to second Nx, animals had received a low-potassium diet (0.04% (weight/weight), Altromin). During surgery for the second Nx and 6 h after second Nx, animals had received 0.4 ml NaHCO₃ by intraperitoneal injection.

In some experiments, growth of animals and daily food intake were monitored. No differences between experimental groups were noted.

[^1H]-1,25(OH)₂D₃ binding capacity

Specific binding of 1,25(OH)₂D₃ in intestinal mucosa was measured exactly as described previously [13]. In brief, the tissue was harvested and processed at 4 °C in TEDMo buffer (10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 10 mM sodium molybdate, pH 7.4; Sigma, Munich, Germany) containing 300 mM phenylmethylsulphonyl fluoride (PMSF, Serva, Heidelberg, Germany) and 500 KIU/ml aprotinin (Serva). Aliquots of a chromatin extract were incubated with 0.2–5.0 nM [3H]-1,25(OH)₂D₃ (26,27-methyl[^1H]) D₃ (specific activity 176 Ci/mmol, Amersham, Braunschweig, Germany) for total binding and 200 times excess unlabelled hormone for unspecific binding. Bound activity was separated from free activity by batchwise treatment with hydroxylapatite (Sigma). Maximal specific binding and affinity of VDR for ligand were calculated by a Scatchard plot.

RNA measurements

Intestinal VDR mRNA concentration was measured by Northern blot analysis exactly as described before [13]. The blots were hybridized with a full-length cDNA probe for human VDR [15] which was a generous gift of Dr W. Pike, Baylor College of Medicine, Houston, USA. A human full-length β-actin cDNA was from Clontech, Palo Alto, USA.

Intestinal 25(OH)D₃-24-hydroxylase activity

The measurement of 24-hydroxylase activity according to a modified protocol adapted from Goff et al. [16] has been described in detail before [13].

Other measurements

Serum creatinine, inorganic phosphate, total protein, and alkaline phosphatase were measured by autoanalyser technique (Hitachi 705 Automatic Analyzer). Serum calcium was measured by flame absorption spectrophotometry (Eppendorf FM, Fa. Eppendorf Gerätebau, Hamburg, Germany). Protein concentrations were quantitated according to Lowry.

Measurement of 1,25(OH)₂D₃ serum concentration was carried out by a scintillation proximity assay after extraction and isolation of 1,25(OH)₂D₃ from serum by Extrelut® columns (Merck, Darmstadt, Germany) and Sep-Pak® silica cartridges (Waters, Bad Homburg, Germany, [17]). In some experiments, 25-hydroxyvitamin D₃ (25(OH)D₃) serum concentrations were determined by a previously described method [18]. No difference between groups was found (data not shown).

Statistical analysis

Data are expressed as means ± SE. Differences between two groups were analysed by Mann–Whitney U test. Differences between several groups were analysed by ANOVA, followed by Duncan’s test.

Results

Serum measurements

Serum measurements in sham-operated intact animals (Intact), in subtotaly nephrectomized rats (Nx) and in totally nephrectomized animals (TNx) are summarized in Table 1. Except in TNx, the serum calcium decreased in parathyroidec tomized animals, whereas serum phosphate rose in those animals. The administration of PTH resulted in increased serum calcium and in decreased serum phosphate. The serum concentration of 1,25(OH)₂D₃ was comparable between experimental groups except in that group which had received 1,25(OH)₂D₃. The markedly elevated serum phosphate in TNx animals was not modulated by changes in parathyroid status.

Effect of parathyroidec tomy on intestinal 1,25(OH)₂D₃ binding capacity

The effect of parathyroidec tomy (PTx) on maximal 1,25(OH)₂D₃ binding capacity in intestinal mucosa was first assessed in Intact. Data from four independent experiments (Figure 1) showed that resection of parathyroid glands did not result in a significant change of intestinal 1,25(OH)₂D₃ binding capacity (493 ± 74 fmol/mg protein in Intact versus 443 ± 51 in Intact-PTx). In contrast to Intact, PTx led to a significant (P < 0.05) decrease in intestinal 1,25(OH)₂D₃ binding capacity in Nx (Figure 1). As compared to subtotal Nx, the mean reduction of intestinal VDR in Nx-PTx was 45% (757 ± 95 fmol/mg protein in Nx versus 417 ± 42 in Nx-PTx).
Table 1. Serum measurements

<table>
<thead>
<tr>
<th>(A) Intact</th>
<th>Intact–Vehicle (n = 12)</th>
<th>Intact–PTx (n = 7)</th>
<th>Intact–PTH (n = 5)</th>
<th>Intact–PTx + D125 (n = 3)</th>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>0.39 ± 0.02</td>
<td>0.40 ± 0.02</td>
<td>0.40 ± 0.01</td>
<td>0.38 ± 0.02</td>
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<tr>
<td>Calcium (mmol/l)</td>
<td>2.43±0.11</td>
<td>1.76±0.10*</td>
<td>2.44±0.07</td>
<td>1.99±0.03</td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>2.65±0.14c</td>
<td>3.51±0.19*</td>
<td>2.74±0.10</td>
<td>3.29±0.21</td>
</tr>
<tr>
<td>1,25(OH)2D3 (pg/ml)</td>
<td>65±5</td>
<td>46±5</td>
<td>70±20</td>
<td>n.d.</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>438±29</td>
<td>375±30b</td>
<td>496±18</td>
<td>416±32</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>54±1</td>
<td>57±2</td>
<td>56±4</td>
<td>56±2</td>
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(B) Subtotal Nx | N x–vehicle (n = 16) | N x–PTx (n = 10) | N x–PTH (n = 5) | N x–PTx + PTH (n = 3) | N x–PTx + D125 (n = 3) |
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<tr>
<td>Creatinine (mg/dl)</td>
<td>1.21±0.09</td>
<td>1.33±0.11</td>
<td>1.05±0.11</td>
<td>1.29±0.13</td>
<td>1.18±0.09</td>
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<tr>
<td>Calcium (mmol/l)</td>
<td>2.46±0.07</td>
<td>2.08±0.06a</td>
<td>2.53±0.04a</td>
<td>2.31±0.07</td>
<td>2.23±0.08</td>
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<tr>
<td>Phosphate (mmol/l)</td>
<td>2.10±0.13</td>
<td>2.31±0.21</td>
<td>2.31±0.13</td>
<td>2.23±0.12</td>
<td>2.59±0.20a</td>
</tr>
<tr>
<td>1,25(OH)2D3 (pg/ml)</td>
<td>33±5</td>
<td>39±19</td>
<td>26±4</td>
<td>38±12</td>
<td>118±40b</td>
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<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>427±27</td>
<td>39±17</td>
<td>516±34d</td>
<td>430±29</td>
<td>341±41</td>
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<tr>
<td>Total protein (g/l)</td>
<td>58±2</td>
<td>56±2</td>
<td>58±4</td>
<td>58±3</td>
<td>56±3</td>
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C: Total–Nx | TNx | TNx–PTx | TNx–PTH |
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<tr>
<td>Creatinine (mg/dl)</td>
<td>2.01±0.15</td>
<td>1.96±0.10</td>
<td>1.99±0.11</td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
<td>2.53±0.14</td>
<td>2.22±0.16</td>
<td>2.39±0.29</td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>5.31±0.67</td>
<td>4.01±0.83</td>
<td>4.15±0.39</td>
</tr>
<tr>
<td>1,25(OH)2D3 (pg/ml)</td>
<td>103±31</td>
<td>119±34</td>
<td>124±30</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>402±101</td>
<td>413±62</td>
<td>349±68</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>52±3</td>
<td>50±5</td>
<td>49±4</td>
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Data are means±SE. Panels A and B: n = number of independent experiments; in each experiment 4–6 data points per group for creatinine, calcium, phosphate, alkaline phosphatase and total protein. For 1,25(OH)2D3, 12–15 measurements per group. Panel C: 12 measurements per group.

Panel A (Intact): a, P<0.01 versus vehicle; b, P<0.05 versus PTH; c, P<0.05 versus PTx-D125. Panel B (Subtotal N x): a, P<0.01 versus vehicle and versus PTH; b, P<0.05 versus PTx + D125; c, P<0.05 versus all other groups; d, P<0.05 versus PTx and PTx-D125. Subtotal N x, subtotal nephrectomy; TNx, total nephrectomy; PTx, parathyroidectomy; + PTH, administration of PTH + D125, administration of 1,25(OH)2D3; nd., not done.
Effect of PTH on intestinal $1,25\text(OH)_2\text{D}_3$ binding capacity

Intestinal $1,25\text(OH)_2\text{D}_3$ binding capacity remained unchanged when PTH was administered either by continuous infusion for 6 days or by bolus injection to Intact (Figure 2). In those experiments, mean $1,25\text(OH)_2\text{D}_3$ binding capacity was $451 \pm 39$ fmol/mg protein in Intact and $429 \pm 17$ in Intact-PTH.

While PTx caused downregulation of intestinal $1,25\text(OH)_2\text{D}_3$ binding capacity in Nx, administration of PTH to Nx (either by continuous infusion for 6 days or by bolus injection) had no effect on intestinal $1,25\text(OH)_2\text{D}_3$ binding capacity in Nx (Figure 2). Mean
Modulation of vitamin D receptor in vivo in uraemia

1,25(OH)₂D₃ binding capacity was 592±47 fmol/mg protein in Nx and 588±48 in Nx-PTH.

Effect of parathyroidectomy and PTH on intestinal vitamin D receptor expression in Nx

Specific 1,25(OH)₂D₃ binding capacity. In further experiments (n=3), the effect of administration of PTH to Nx-PTx was assessed. Mean intestinal 1,25(OH)₂D₃ binding capacity in Nx was 700±71 fmol/mg protein; it was 423±31 in Nx-PTx (P<0.05). Administration of PTH by continuous infusion (starting immediately after PTx) to Nx-PTx significantly (P<0.05) augmented intestinal 1,25(OH)₂D₃ binding capacity to 790±113 (Figure 3). The Kd values were comparable between the experimental groups (Nx 0.52±0.11 nM; Nx-PTx 0.56±0.10 nM; Nx-PTx+PTH 0.61±0.10).

In an additional experiment, intestinal 1,25(OH)₂D₃ binding capacity in Nx at 6 days after surgery was 740±56 fmol/mg protein. In Nx-PTx, maximal 1,25(OH)₂D₃ binding capacity had decreased to 374±45 fmol/mg protein (P<0.05 versus Nx). Infusion of PTH for 48 h starting 6 days after Nx-PTx elevated 1,25(OH)₂D₃ binding capacity to a range which was comparable to that in Nx (775±116 fmol/mg protein (P<0.05 versus Nx). Thus the stimulatory effect of PTH on 1,25(OH)₂D₃ binding capacity in Nx-PTx was still present, even if PTH administration was begun several days after PTx.

Vitamin D receptor mRNA concentration. VDR mRNA concentrations in intestinal mucosa were measured by Northern blot. Data from a representative experiment are shown in Figure 4. As compared to subtotal Nx, the VDR mRNA concentration (normalized versus β-actin) in Nx-PTx was decreased by 20% in this experiment. By infusion of PTH in Nx-PTx, the VDR mRNA concentration was restored to a similar value as in subtotal Nx. The VDR mRNA results which were confirmed in additional experiments are summarized in Table 2. Taken together, intestinal VDR mRNA concentration fell slightly by a mean of 23% in Nx-PTx versus Nx (as determined in three separate experiments).

Effect of 1,25(OH)₂D₃ on intestinal 1,25(OH)₂D₃ binding capacity in PTx

In additional experiments, the effect of the administration of 1,25(OH)₂D₃ on intestinal 1,25(OH)₂D₃ bind-

Fig. 3. Effect of parathyroidectomy and PTH on intestinal 1,25(OH)₂D₃ binding capacity. PTH (100 ng/kg body weight/day) or vehicle were infused continuously for 6 days until sacrifice of animals. Data from three independent experiments is shown. Per experiment, measurements were made in duplicate or triplicate from tissue pools of 2–5 tissues. Nx, subtotal nephrectomy; PTx, parathyroidectomy; PTH, administration of PTH. P<0.05 for Nx-PTx versus Nx and versus Nx-PTx-PTH.

Fig. 4. Northern blot analysis of poly(A⁺) RNA (5 µg/lane) from intestinal mucosa of Nx (pooled tissue from five animals per group). Tissues were examined 6 days after the second nephrectomy. The blot was sequentially hybridized with a ³²P-labelled cDNA probe for vitamin D receptor (upper panel) and a ³²P-labelled cDNA probe for β-actin (lower panel). Lane 1: Nx-PTx, VDR/β-actin ratio 1.28. Lane 2: Nx, 1.59. Lane 3: Nx-PTx+PTH, 1.81. PTx, parathyroidectomy. +PTH, administration of PTH (100 ng/kg bodyweight/day for 6 days by osmotic mini pump).

Table 2. VDR/β-actin mRNA expression in intestinal mucosa in Nx

<table>
<thead>
<tr>
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<th>VDR/β-actin mRNA ratio</th>
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<tbody>
<tr>
<td>Nx</td>
<td>100</td>
</tr>
<tr>
<td>Nx-PTx</td>
<td>77.3±7.5</td>
</tr>
<tr>
<td>Nx-PTx+PTH</td>
<td>99.1±6.3</td>
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</table>

The data shown was from three independent experiments. Per experiment, mRNA concentration (5 µg poly(A⁺)-enriched RNA) was measured in triplicate from tissue pools of 2–5 intestinal mucosa samples. VDR/β-actin ratio of Nx was set as 100. Statistical analysis was by ANOVA. Nx, subtotal nephrectomy; PTx, parathyroidectomy; +PTH, PTH administration. Nx-PTx, P<0.05 versus other groups.
ing capacity was assessed in PTx animals. Both in Intact PTx and in Nx PTx, the 1,25(OH)\(_2\)D\(_3\) significantly elevated 1,25(OH)\(_2\)D\(_3\) binding capacity (Figure 5). In Nx-PTx it was elevated to a concentration, which was similar to that in Nx.

**Effect of parathyroidectomy and PTH on intestinal 1,25(OH)\(_2\)D\(_3\) binding capacity in totally nephrectomized rats**

An experiment with totally nephrectomized rats (TNx) was carried out to assess the possibility that the stimulatory effect of PTH on intestinal 1,25(OH)\(_2\)D\(_3\) binding capacity in Nx-PTx was mediated through stimulation of 1,25(OH)\(_2\)D\(_3\) production in remnant kidney of Nx. Serum measurements in TNx are listed in Table 1. Intestinal 1,25(OH)\(_2\)D\(_3\) binding capacity in TNx was 540±99 fmol/mg protein (n=3 pools of four tissues each). Parathyroidectomy of TNx resulted in a lower 1,25(OH)\(_2\)D\(_3\) binding capacity (389±67, P<0.05 versus TNx). The stimulatory effect of PTH on intestinal 1,25(OH)\(_2\)D\(_3\) binding capacity was maintained in parathyroidectomized TNx. Injection of PTH resulted in an approximately 1.8-fold increase in intestinal 1,25(OH)\(_2\)D\(_3\) binding capacity to 708±54 (P<0.05 versus TNx-PTx).

**Effect of parathyroidectomy on induction of intestinal 25(OH)D3-24-hydroxylase activity by 1,25(OH)\(_2\)D\(_3\)**

The stimulation of 24,25(OH)\(_2\)D\(_3\) production by 1,25(OH)\(_2\)D\(_3\) is a measure of the biological responsiveness of a tissue to 1,25(OH)\(_2\)D\(_3\). To test if decreased intestinal 1,25(OH)\(_2\)D\(_3\) binding capacity in Nx-PTx versus Nx was associated with decreased inducibility of 24-hydroxylase, intestinal 24,25(OH)\(_2\)D\(_3\) production was measured in subtotally nephrectomized rats with and without parathyroidectomy six h after intraperitoneal injection of 2.5 nmol 1,25(OH)\(_2\)D\(_3\). 24,25(OH)\(_2\)D\(_3\)-24-Hydroxylase activity was undetectable both in vehicle-treated Nx and in vehicle-treated Nx-PTx. 24-Hydroxylase activity was induced in both groups which had received 1,25(OH)\(_2\)D\(_3\). The 1,25(OH)\(_2\)D\(_3\)-stimulated intestinal 24,25(OH)\(_2\)D\(_3\) production by Nx was 385±81 pmol/mg protein/30 min (Table 3). This was significantly higher (P<0.01) than 24,25(OH)\(_2\)D\(_3\)-production by Nx-PTx.

**Table 3. Induction of intestinal 25(OH)D3-hydroxylase activity by 1,25(OH)\(_2\)D\(_3\) in Nx. Effect of PTx**

<table>
<thead>
<tr>
<th></th>
<th>Nx + D125</th>
<th>Nx-PTx + D125</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDR (fmol/mg protein)</td>
<td>457±42</td>
<td>331±48</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>24-Hydroxylase</td>
<td>385±81</td>
<td>209±68</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(pmol/mg protein/30 min)</td>
<td>2.57±0.02</td>
<td>2.00±0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum calcium (mmol/l)</td>
<td>2.57±0.02</td>
<td>2.00±0.10</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data shown is from duplicate measurements of VDR and 24-hydroxylase in five tissue pools per group (two animals per tissue pool). Measurements were made 6 h after administration of 2.5 nmol 1,25(OH)\(_2\)D\(_3\). Serum calcium: n=20. 24-Hydroxylase activity was not detectable in vehicle-treated Nx. VDR, intestinal 1,25(OH)\(_2\)D\(_3\) binding capacity; Nx, subtotal nephrectomy; PTx, parathyroidectomy; D125, 1,25(OH)\(_2\)D\(_3\); P, significance level.
Available data show that the rat renal and cardiac 1,25(OH)₂D₃ binding capacity (Table 3).

**Discussion**

The present study assessed regulation of intestinal VDR by PTH in rats with normal kidney function (Intact) and in uraemic animals (Nx). In agreement with an earlier study [3], administration of PTH to control animals did not modulate intestinal 1,25(OH)₂D₃ binding capacity. Similarly, PTH did not influence intestinal 1,25(OH)₂D₃ binding capacity in Nx. However, the response to PTx in terms of intestinal 1,25(OH)₂D₃ binding capacity was different between Intact and Nx. The 1,25(OH)₂D₃ binding capacity did not change in Intact after PTx whereas intestinal 1,25(OH)₂D₃ binding capacity and (to a lesser degree) intestinal VDR mRNA concentration decreased in Nx after PTx. This decrease was reversible when PTH was given to Nx-PTx whereas administration of PTH to parathyroidectomized animals had no effect on intestinal 1,25(OH)₂D₃ binding capacity in Intact.

The reduction of intestinal 1,25(OH)₂D₃ binding capacity and VDR mRNA concentration in Nx-PTx indicated that functioning parathyroid glands were required for maintenance of VDR expression in renal failure. In contrast, functioning parathyroid glands had no role in the maintenance of intestinal VDR expression in animals with intact kidney function. Thus the data of this study further underscored differential regulation of intestinal VDR expression in Intact versus Nx under certain conditions, i.e. in parathyroidectomized animals. In contrast, no difference was found between Nx and Intact when PTH was given to animals with intact parathyroid glands; the excess PTH had no effect on intestinal VDR expression.

The mechanism for downregulation of intestinal 1,25(OH)₂D₃ binding capacity in Nx-PTx was not evident. One could have assumed that PTH was involved in the maintenance of intestinal 1,25(OH)₂D₃ binding capacity by a direct effect on the intestinal cell. This possibility was not excluded a priori since the PTH/PTHrP receptor mRNA is expressed with low intensity in the ileum of normal rats [19], and since the decrease in serum PTH after PTx could have resulted in diminished biological activity of PTH. However, parathyroidectomy of animals with intact parathyroid glands did not influence intestinal 1,25(OH)₂D₃ binding capacity rendering a direct relationship between a decrease of serum PTH and a decrease of vitamin D receptor expression unlikely. Additional factors which could be involved in the modulation of intestinal 1,25(OH)₂D₃ binding capacity in uraemia are changes in PTH/PTHrP receptor expression and altered responsiveness to PTH. Available data show that the rat renal and cardiac PTH/PTHrP receptors are downregulated in renal failure [20,21], and that parathyroectomy of uraemic rats does not further influence renal PTH/PTHrP receptor expression [22]. To our knowledge, comparable data for the intestinal PTH/PTHrP receptor do not exist. Thus it is not clear whether the decrease of VDR expression in Nx-PTx is the result of a diminished direct effect of PTH on the intestinal cell.

Similarly, the mechanism of the PTH-mediated increase of intestinal 1,25(OH)₂D₃ binding capacity in Nx-PTx was indeterminate. One possibility was that a transient increase in serum 1,25(OH)₂D₃ (through stimulation of renal 1,25(OH)₂D₃ synthesis by PTH) was involved in upregulation of intestinal VDR. We had shown in this study that administration of 1,25(OH)₂D₃ to Nx-PTx led to a marked increase in intestinal 1,25(OH)₂D₃ binding capacity. To examine a possible indirect role of 1,25(OH)₂D₃ in PTH-mediated receptor regulation in Nx-PTx, totally nephrectomized animals were studied. In that experiment, the injection of PTH caused upregulation of intestinal 1,25(OH)₂D₃ binding capacity in totally nephrectomized and parathyroidectomized animals, demonstrating that the PTH-mediated rise of intestinal 1,25(OH)₂D₃ binding capacity in Nx-PTx had to be independent from changes in 1,25(OH)₂D₃ serum concentration. In theory, extrarenally synthesized 1,25(OH)₂D₃ (i.e. by monocytes/macrophages) could also have caused an increase in serum 1,25(OH)₂D₃.

In our opinion, that possibility can be virtually ruled out. Firstly, PTH did not stimulate 1,25(OH)₂D₃ synthesis by extrarenal sources (i.e. haematopoietic cells, [23]). Secondly, an increase in serum 1,25(OH)₂D₃ through extrarenal synthesis in vivo in uraemia was only observed when pharmacological amounts of 25(OH)D₃ were used [24]. That was not the case in our study.

A number of studies in vitro [25,26] and in vivo [20] had shown that excess PTH resulted in downregulation of the PTH/PTHrP receptor and desensitization to PTH. Those results argued against a direct stimulation by PTH of intestinal VDR expression in Nx-PTx. Moreover, PTH had no effect on 1,25(OH)₂D₃ binding capacity in animals with intact renal function (independent from parathyroid status) and in uraemic animals with functioning parathyroid glands. Our results suggested that one or more additional factors had to be involved in the regulation of VDR by PTH in Nx-PTx.

Those additional factors could have included altered serum concentrations of calcium, phosphate, and 1,25(OH)₂D₃. Mean serum calcium was significantly reduced in Nx-PTx as compared to Nx, whereas serum calcium had increased in Nx-PTx after PTH administration. Thus, the changes in serum calcium paralleled changes in VDR expression under those experimental conditions. However, VDR and serum calcium were discordant under other experimental conditions. Serum calcium was low in Intact-PTx, but intestinal 1,25(OH)₂D₃ binding capacity was unchanged. Moreover, administration of 1,25(OH)₂D₃ to Nx-PTx upregulated intestinal 1,25(OH)₂D₃ binding capacity
whereas serum calcium was comparable between the two groups. Those results ruled out a direct role of extracellular calcium concentration in regulation of intestinal VDR. Likewise, neither serum phosphate nor serum 1,25(OH)2D3 were consistently correlated with intestinal 1,25(OH)2D3 binding capacity under the experimental conditions of this study.

Several studies showed a correlation between VDR concentration and biological responsiveness to 1,25(OH)2D3 in various vitamin D target tissues [27–29]. To examine this point, 1,25(OH)2D3-stimulated 25(OH)D3-24-hydroxylase activity was measured in intestinal mucosa of Nx versus Nx-PTx. The induction of 24-hydroxylase activity was indeed significantly higher in intestinal mucosa of Nx with functioning parathyroid glands as compared to parathyroidectomized Nx. One could argue that acute changes in 1,25(OH)2D3 binding capacity (i.e., up- or downregulation between injection of 1,25(OH)2D3 and harvest of tissues 6 h later) might have influenced the outcome of the experiment. However, the data in Figure 5 [1,25(OH)2D3-induced upregulation of VDR in Nx-PTx] showed that such an effect would have resulted in an underestimation of the difference between the two groups.

Thus, the decrease in intestinal 1,25(OH)2D3 binding capacity in parathyroidectomized Nx was, under our experimental conditions, functionally significant with respect to induction of 24,25(OH)2D3 production by 1,25(OH)2D3.

Taken together, we show that PTH is involved in regulation of intestinal VDR under certain conditions in experimental uremia whereas PTH has no effect on intestinal VDR in animals with intact kidney function. This further underscores the altered regulation of VDR expression in uremia. In a clinical context, one could assume that VDR is downregulated in parathyroidectomized individuals resulting in decreased responsiveness to 1,25(OH)2D3.

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