Regulation of vitamin D-1α-hydroxylase and -24-hydroxylase expression by dexamethasone in mouse kidney

N Akeno, A Matsunuma, T Maeda, T Kawane and N Horiuchi

Department of Biochemistry, Ohu University School of Dentistry, Koriyama 963–8611, Japan

(Requests for offprints should be addressed to N Horiuchi; E-mail: fwga4746@mb.infoweb.ne.jp)

Abstract

We investigated the effects of dexamethasone on vitamin D-1α-hydroxylase and -24-hydroxylase expression and on vitamin D receptor (VDR) content in the kidneys of mice fed either a normal (NCD) diet or a calcium- and vitamin D-deficient (LCD) diet for 2 weeks. For the last 5 days mice received either vehicle or dexamethasone (2 mg/kg per day s.c.). Dexamethasone significantly increased plasma calcium concentrations without changing plasma concentrations of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) in both NCD and LCD groups. Northern blot and enzyme activity analyses in NCD mice revealed that dexamethasone increased renal VDR mRNA expression modestly and greatly increased 24-hydroxylase mRNA abundance and enzyme activity, but did not affect 1α-hydroxylase mRNA abundance and enzyme activity. In mice fed an LCD diet, dexamethasone increased renal VDR mRNA expression 1.5-fold, decreased 1α-hydroxylase mRNA abundance (52%) and activity (34%), and markedly increased 24-hydroxylase mRNA abundance (16-fold) and enzyme activity (9-fold). Dexamethasone treatment did not alter functional VDR number (Bmax 125–141 fmol/mg protein) or ligand affinity (Kd 0.13–0.10 nM) in LCD mice. Subcutaneous injections of 1,25(OH)2D3 (0.24 nmol/kg per day for 5 days) into NCD mice strongly increased renal 24-hydroxylase mRNA abundance and enzyme activity, while there was no effect of dexamethasone on renal 24-hydroxylase expression in these mice. This may be due to overwhelming induction of 24-hydroxylase by 1,25(OH)2D3. These findings suggest that glucocorticoid-induced osteoporosis is caused by direct action of the steroids on bone, and the regulatory effect of glucocorticoids on renal 25-hydroxyvitamin D3 metabolism may be less implicated in the initiation and progression of the disease.


Introduction

The 1932 description of glucocorticoid excess by Harvey Cushing suggested symptomatic osteoporosis as a clinical feature (Cushing 1932). Increased concentrations of glucocorticoids are thought to alter bone remodeling by decreasing bone formation and increasing bone resorption, resulting in a net loss of bone volume in humans and animals (Lukert & Raisz 1990, Reid 1997). Furthermore, glucocorticoids decrease net intestinal calcium absorption (Klein et al. 1977, Aloia et al. 1984), although details of the mechanism are still unclear. Glucocorticoids have been shown to increase renal calcium excretion (Suzuki et al. 1983, Reid & Ibbertson 1987, Cosman et al. 1994). Therefore, the net effect of glucocorticoid in intestine and kidney results in a negative calcium balance which leads to secondary hyperparathyroidism, as evidenced by increased immunoreactive parathyroid hormone (PTH) in steroid-treated patients (Suzuki et al. 1983). Glucocorticoids elicit phosphaturia and decrease the tubular reabsorption of phosphate (Cosman et al. 1994). A dexamethasone-induced decrease in the maximum velocity (Vmax) of phosphate transport across the renal brush border membrane correlates with reductions in renal mRNA coding for the sodium gradient-dependent phosphate transporter and in renal content of the protein (Levi et al. 1995). Thus, glucocorticoids have direct effects on the kidney, although overall renal effects include results of secondary hyperparathyroidism.

Metabolism of 25-hydroxyvitamin D3 (25OHD3), a circulating form of vitamin D3, occurs mainly in the kidney where it is converted into 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), the active form of the vitamin, and 24,25-dihydroxyvitamin D3 (24,25(OH)2D3), a catabolic product and putative regulator of bone formation (DeLuca 1988, Ono et al. 1996, Haussler et al. 1998). The synthesis of these substances is reciprocally regulated by a number of factors including 1,25(OH)2D3 and PTH (DeLuca 1988, Haussler et al. 1998). The vitamin D-endocrine system is the major regulator of mineral ion metabolism, while glucocorticoids also affect such metabolism, so suspicion has arisen that glucocorticoids can influence 25OHD3
metabolism in the kidney. However, studies attempting to demonstrate such an effect have yielded conflicting results. Chronic glucocorticoid excess has had varied reported effects on plasma 1,25(OH)2D3 concentrations, including increases (Bikle et al. 1993, Cosman et al. 1994), no change (Seeman et al. 1980, Prummel et al. 1991), and decreases (Chesney et al. 1978, Morris et al. 1990). Moreover, whether chronic glucocorticoid excess influences plasma concentrations of 24,25(OH)2D3 is not known. Clearance rates of 1,25(OH)2D3 have been found to be normal in humans receiving glucocorticoids (Seeman et al. 1980), although the steroids accelerate degradation of 1,25(OH)2D3 in intestinal mucosa (Carré et al. 1974). Intestinal receptors for 1,25(OH)2D3 have been reported to be increased in rats (Hirst & Feldman 1982a) by glucocorticoids, but decreased in mice (Hirst & Feldman 1982b). These conflicting results involving the complexes of vitamin D metabolism accentuate the importance of defining regulatory effects of glucocorticoids on the expression of 1α- and 24-hydroxylases in the kidney.

Genes encoding vitamin D-1α-hydroxylase, an enzyme responsible for 1,25(OH)2D3 synthesis, and 24-hydroxylase, an enzyme responsible for production of 24,25(OH)2D3 and 1α,24,25-tri hydroxvitamin D3 (1α, 24,25(OH)2D3), have recently been cloned, permitting the hydroxylase mRNAs to be quantified in mice (Akeno et al. 1997, Takeyama et al. 1997). The present study was designed to determine whether dexamethasone affects renal 1α- and 24-hydroxylase gene expression and activity in mice. We found that dexamethasone modestly decreases renal 1α-hydroxylase expression in mice fed a low calcium and vitamin D-deficient (LCD) diet, while clearly increasing 24-hydroxylase gene expression and activity in the kidneys of mice fed either normal (NCD) or LCD diets.

Materials and Methods

Diets and animals

Male ddY mice (8 weeks old) were purchased from SLC (Hamamatsu, Japan). They were fed either a normal diet (rodent chow containing 1.6 IU vitamin D3/g chow, 1.42% calcium and 1.16% phosphorus) or a low calcium and vitamin D-deficient diet containing 0.01% calcium and 0.3% phosphorus (AIN-93 G minus calcium and vitamin D) for 2 weeks (Reeves et al. 1993a, b). Both diets were obtained from Oriental Bio-service Co. (Tokyo, Japan). The mice receiving a normal diet were designated the NCD group and animals fed a synthetic diet (AIN-93 G minus calcium and vitamin D) were designated the LCD group. Animals received daily injection (s.c.) of either vehicle alone (isotonic saline) or dexamethasone phosphate (Sigma Chemical Co., St Louis, MO, USA) at a dose of 2 mg/kg for 5 days. They were killed by cervical dislocation under anesthesia 24 h after the last administration of dexamethasone. Total plasma calcium and phosphorus levels were measured colorimetrically. Plasma 1,25(OH)2D3 was measured by a radio receptor assay with calf thymus receptor and C18/OH cartridge purification using a 1,25(OH)2D3 kit (a gift from Yamasa Shoyu Co., Choshi, Japan) (Saikatsu et al. 1993). Plasma mouse PTH was determined using the rat PTH IRMA kit (Immunotopics, Inc., San Clemente, CA, USA) (Meyer et al. 1994). Kidneys were removed and either homogenized for determination of 1α-hydroxylase and 24-hydroxylase activities, or rapidly frozen in liquid N2 for isolation of total RNA. The animal studies were approved by the Animal Care and Use Committee of Ohsu University and conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals.

Probes for mRNA determination

The probe used in this study for 24-hydroxylase was a previously cloned 3·3 kb full-length cDNA (Akeno et al. 1997). That for 1α-hydroxylase was 2.4 kb full-length cDNA which we have cloned, and the sequence was identical to that reported by Takeyama et al. (1997). The mouse vitamin D receptor (VDR) probe was a 562 bp cDNA synthesized by RT-PCR and cloned into the pT7Blue T-vector (Invitrogen, San Diego, CA, USA). The probe used in this study for 24-hydroxylase was a previously cloned 3·3 kb full-length cDNA (Akeno et al. 1997). That for 1α-hydroxylase was 2.4 kb full-length cDNA which we have cloned, and the sequence was identical to that reported by Takeyama et al. (1997). The mouse vitamin D receptor (VDR) probe was a 562 bp cDNA synthesized by RT-PCR and cloned into the pT7Blue T-vector (Invitrogen, San Diego, CA, USA). The nucleotide sequence of the VDR cDNA corresponded to nucleotide numbers 486–1047 in the report of Kamei et al. (1995). Probes were labeled with [α-32P]dCTP (110 TBq/mmol; ICN Biochemicals, Costa Mesa, CA, USA) by the random oligopriming method using a Megaprime DNA labeling kit (Pharmacia Biotech UK Ltd., Bucks, UK). The cyclophilin cDNA probe was used to control loading of RNA on the gels and transfer onto membranes.

Northern blot analysis

Total RNA was extracted from kidney cortex using guanidine thiocyanate as described previously (Akeno et al. 1997). Total RNA was fractionated in 1% agarose gels containing formaldehyde and transferred onto Hybond-N+ membranes (Amersham International plc). The membranes were hybridized with mouse 1α-hydroxylase, 24-hydroxylase, VDR or cyclophilin cDNA probes that had been labeled with [α-32P]dCTP by the Megaprime DNA labeling system. Hybridization was performed for 2 days at 42 °C in 50% formamide, 5 × Denhardt’s solution, 0·5% SDS, and 5 × SSPE (150 mM NaCl, 10 mM sodium phosphate, and 1 mM EDTA), after which the membranes were washed in 0·1 × SSPE–0·1% SDS at 65 °C for 15 min and exposed to Hyperfilm at −80 °C with intensifying screens. The amounts of mRNA were quantified by densitometric scanning of the autoradiograms, and the abundance of mRNAs of interest was normalized relative to cyclophilin mRNA.


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Measurement of renal hydroxylase activities

The kidney cortex was minced, then washed in ice-cold homogenization buffer (0.1 M sucrose, 25 mM sodium succinate, 2 mM MgCl₂, 1 mM EDTA, 20 mM Tris–Hepes, pH 7.4), and homogenized in the same solution (20 ml/g tissue). For measurement of 1α-hydroxylase activity, 4 µg 25OHD₃ (Phillips Duphar, Amsterdam, The Netherlands), dissolved in 10 µl ethanol, was added to 1 ml 5% homogenate, and the mixture was incubated at 37 °C for 30 min. The reactions were stopped by the addition of 1 ml acetonitrile. 1,25(OH)₂D₃ synthesis was quantified using a 1,25(OH)₂D₃ kit from Yamasa Shoyou Co. Data are expressed as fmol/mg protein per min. Because 1α-hydroxylase activity was very low, we used the sensitive method of Lobaugh & Drezner (1983) who showed that in vitro production of 1,25(OH)₂D₃ by mouse kidney homogenates can be measured accurately and at low concentration. This method indicated the Vₘₐₓ of the 1α-hydroxylase activity. For 24-hydroxylase activity assay, tritiated 1,25(OH)₂D₃ (250 pmol, 50 000 c.p.m.) from Dupont NEN (Boston, MA, USA) was used as the substrate and incubation was performed at 37 °C for 15 min using 1 ml 5% kidney homogenate. The reactions were stopped by the addition of 1 ml acetonitrile. Vitamin D metabolites were extracted by C18/Sep-Pak (Waters, Milford, MA, USA) and separated by high-performance liquid chromatography as described previously (Akeno et al. 1997). Activity of 24-hydroxylase is expressed in fmol/mg protein per min.

Quantitation of specific 1,25(OH)₂D₃ binding

Soluble fraction of kidney cortex was prepared as previously described (Saikatsu et al. 1993). This fraction contained total VDR in the tissue. All steps were carried out at 4 °C. Briefly, mouse kidneys were homogenized (20% w/v) in the assay buffer containing 50 mM Tris–HCL, 500 mM KCl, 5 mM dithiothreitol, 10 mM Na₂MoO₄ and 1:5 mM EDTA, pH 7.5 by a polytron PT10–35 tissue disrupter (Kinematica AG, Lucerne, Switzerland). The homogenates were centrifuged for 60 min at 300 000 g. The supernatant (cytosol) was removed and fractionated with solid (NH₄)₂SO₄ (enzyme grade) to 35% saturation. The soluble fraction was centrifuged for 30 min at 3000 r.p.m. The supernatant was discarded, and the pellets were frozen by liquid N₂ and stored at −80 °C until used for the ligand-binding study. Stock receptor (pellets) was diluted with the assay buffer (dilution 1:10), and 350 µl receptor solution was incubated with various concentrations of tritiated 1,25(OH)₂D₃ in the presence or absence of 500 ng unlabeled 1,25(OH)₂D₃ for 16 h at 4 °C. Separation of bound from free hormone was achieved by the addition of 150 µl dextran-coated charcoal suspension. The protein in the preparations was measured. Results are expressed as fmol of 1,25(OH)₂D₃ receptor/mg protein.

Statistical analysis

The data are described as means ± s.e.m. Differences between treated and untreated groups were assessed by Student’s t-test. Multiple comparisons were evaluated by ANOVA followed by Fisher’s protected least significant difference. Statistical analysis was performed with the Statview 4.02 software package (Abacus Concepts Inc., Berkeley, CA, USA). A P value of <0.05 was considered statistically significant.

Results

Effects of dexamethasone on plasma calcium, phosphorus, and 1,25(OH)₂D₃

Mice were injected s.c. with dexamethasone (2 mg/kg per day s.c.) for 5 successive days, and the plasma parameters were measured 24 h after the last injection of steroid. Dexamethasone significantly increased plasma calcium in both groups (NCD and LCD) (Table 1). The plasma phosphorus concentration decreased with administration of dexamethasone in NCD mice, but did not change in LCD mice. Calcium restriction by the LCD diet resulted in a significant decrease in plasma calcium and a significant increase in immunoassayable plasma PTH values. Dexamethasone markedly reduced plasma PTH concentrations coincident with an elevation in plasma calcium concentrations in the LCD group, but did not affect PTH levels in NCD mice. We also examined the effect of dexamethasone on plasma 1,25(OH)₂D₃ concentration. Dexamethasone did not significantly change plasma 1,25(OH)₂D₃ concentrations from control values in either dietary group. However, feeding the LCD diet for 2 weeks significantly increased the plasma concentrations of 1,25(OH)₂D₃ over those in the NCD group (Table 1).

Effects of dexamethasone on renal 1α-hydroxylase, 24-hydroxylase, and VDR expression in normally fed mice

Total RNA from the kidneys of NCD mice injected with dexamethasone (2 mg/kg per day s.c.) or with vehicle was subjected to Northern blot analysis using VDR, 1α-hydroxylase, and 24-hydroxylase cDNA probes (Fig. 1). Dexamethasone increased VDR mRNA abundance and clearly elevated the tissue content of 24-hydroxylase, and VDR expression in normally fed mice

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NCD mice; these mice expressed only very low constitutive amounts of 1α-hydroxylase message and protein that were not responsive to dexamethasone.

**Effect of dexamethasone in mice fed an LCD diet**

The presence of VDR in mouse kidneys was examined by receptor binding study. Figure 3 depicts the binding of increasing concentrations of [3H]1,25(OH)2D3 in three groups of mice. Figure 3 and Table 2 show that dexamethasone did not significantly alter the number of VDR in LCD mice, and that restricting calcium and vitamin D did not influence the functional receptor expression. The affinity of VDR for 1,25(OH)2D3 was unchanged in these groups (Kd 0·10–0·12 nM). The LCD diet did not, in itself, affect VDR mRNA abundance, but dexamethasone administration modestly elevated VDR expression in mice fed the LCD diet (Fig. 4). We next assessed the effect in vivo of dexamethasone administration on renal 1α-hydroxylase expression in mice (Fig. 5). Renal 1α-hydroxylase mRNA abundance and enzyme activity were markedly increased in mice fed the LCD diet compared with normal mice. Dexamethasone administration at a dose of 2 mg/kg per day led to a modest but significant fall in 1α-hydroxylase mRNA expression and enzyme activity. In contrast, 24-hydroxylase mRNA expression and enzyme activity increased 16- and 9-fold respectively in LCD mice (Fig. 6). In itself, restriction of calcium and vitamin D intake for 2 weeks in mice decreased renal 24-hydroxylase mRNA expression and enzyme activity.

**Effect of dexamethasone on renal 24-hydroxylase expression in vitamin D excess**

We assessed the effect of 1,25(OH)2D3 excess on renal 24-hydroxylase expression. The increase in 24-hydroxylase mRNA abundance and activity occurring with dexamethasone was completely abolished in mice that were also injected with 0·24 nmol/kg 1,25(OH)2D3 for 5 days (Fig. 7). Although no longer responsive to dexamethasone, renal mRNA expression and enzyme activity of 24-hydroxylase were much greater in mice receiving 1,25(OH)2D3 than in those without this treatment (Fig. 7).

**Discussion**

Administration of glucocorticoids such as dexamethasone enhances bone resorption and decreases bone formation to induce osteopenia (Lukert & Raisz 1990), while vitamin D metabolites, especially 1,25(OH)2D3, have important roles in maintaining bone integrity. However, the effects of glucocorticoids on vitamin D metabolism are controversial (Lukert & Raisz 1990, Reid 1997). The present study was undertaken to define how in vivo administration of dexamethasone influences vitamin D metabolism through the expression of 25OHD3–1α-hydroxylase and -24-hydroxylase in the kidney.

Dexamethasone markedly elevates plasma calcium levels in mice fed a normal diet as well as in mice with dietary calcium and vitamin D restriction. The hypercalcaemia induced by a pharmacologic dose of dexamethasone may reflect an overwhelming degree of bone resorption (Gronowicz et al. 1990, Conaway et al. 1996), although glucocorticoids decrease intestinal absorption of calcium (Klein et al. 1977, Aloia et al. 1984) and increase urinary calcium excretion (Suzuki et al. 1983, Reid & Ibbertson 1987, Cosman et al. 1994). Plasma levels of 1,25(OH)2D3 are unlikely to cause the increased concentrations of plasma calcium seen in dexamethasone-treated mice, since steroid administration did not affect the plasma 1,25(OH)2D3 concentrations in normally fed or LCD mice.

Since 1,25(OH)2D3, an important regulator of calcium metabolism, is synthesized in the renal proximal tubules by
the specific enzyme, 1α-hydroxylase (DeLuca 1988, Haussler et al. 1998), monitoring of 1α-hydroxylase gene expression in glucocorticoid-treated animals is of special interest. The recent cloning of a mouse 1α-hydroxylase cDNA (Takeyama et al. 1997) enabled us to compare 1α-hydroxylase mRNA expression with enzyme activity. In normally fed mice, 1α-hydroxylase mRNA expression and enzyme activity was not affected by a pharmacologic dose (2 mg/kg per day) of dexamethasone, because the expression was very low. We then studied mice with restricted calcium and vitamin D intake and consequently elevated 1α-hydroxylase expression. The present study showed that dexamethasone modestly suppressed both mRNA abundance and activity of renal 1α-hydroxylase in such animals. Moreover, glucocorticoids influenced inducible but not basal expression of renal 1α-hydroxylase. Although the glucocorticoid modestly increased VDR gene expression, the steroid did not significantly alter

Figure 1 Northern blot analysis of total RNA from the kidneys of NCD mice. Mice were given a daily s.c. injection of either vehicle or dexamethasone (2 mg/kg) for 5 successive days. Total RNA (20 μg for 24-hydroxylase and VDR; 30 μg for 1α-hydroxylase) was extracted and subjected to Northern blot analysis. Nylon membranes were hybridized with vitamin D receptor, 1α-hydroxylase, 24-hydroxylase and cyclophilin cDNA probes.

Figure 2 Effects of dexamethasone (Dex) on 1α-hydroxylase (A) and 24-hydroxylase (B) expression in the NCD mouse kidney. Mice were injected s.c. with either vehicle or dexamethasone (2 mg/kg) for 5 days and killed 24 h after the last injection. The amounts of hydroxylase mRNA were determined by quantitative Northern blot analysis (dotted bars), and enzyme activity (solid bars) were measured in kidney homogenate as described in Materials and Methods. Data are means ± S.E.M. of five mice.
functional VDR expression and 1,25(OH)\(_2\)D\(_3\) concentrations in plasma. Mild inhibition of 1α-hydroxylase gene expression by dexamethasone would not result from the alteration of activated VDR binding on a putative negatively acting vitamin D-responsive element in a 5′-flanking region of the gene.

In contrast, subcutaneous cortisol injections in chicks reportedly stimulated 1α-hydroxylase activity in renal tubule preparations (Spanos et al. 1977). Further, a more recent clinical study found that pharmacologic doses of glucocorticoid used to treat patients with multiple sclerosis caused increased concentrations of plasma 1,25(OH)\(_2\)D\(_3\).
(Cosman et al. 1994). The basis of the apparent discrepancy between these previous findings (Spanos et al. 1977, Cosman et al. 1994) and ours is unknown. We have shown that the glucocorticoid reduced renal 1α-hydroxylase mRNA abundance and enzyme activity without marked...
changes in plasma phosphate concentration, which is a potent regulator of renal 1α-hydroxylase. This result suggests that glucocorticoids may act directly on the kidney to suppress 1α-hydroxylase expression. An alternative explanation is that during restriction of calcium and vitamin D intake for 2 weeks mice develop hypocalcemia associated with hyperparathyroidism; these abnormalities are decreased when dexamethasone increases bone resorption. Since PTH is a principal stimulator of 1α-hydroxylase activity (Horiuchi et al. 1977), glucocorticoid-induced suppression of renal 1α-hydroxylase expression in LCD mice is likely to have resulted from a decrease in plasma PTH concentration. Also, it is possible that the elevation of plasma calcium concentrations by glucocorticoid treatment suppresses the 1α-hydroxylase expression in the kidney (Trechsel et al. 1980). In contrast, dexamethasone administration to NCD mice did not affect 1α-hydroxylase expression. This finding is explained by the low oscillation of plasma PTH concentrations in mice treated with dexamethasone and vehicle.

Although many investigators have indicated that 24-hydroxylase gene is activated by the binding of 1,25(OH)₂D₃–VDR complexes on the gene promoter (Ohyama et al. 1994, Zierold et al. 1994), the effect of dexamethasone on 24-hydroxylase gene expression is not known. The present studies have clearly demonstrated that dexamethasone administration caused a large increase in renal mRNA abundance and enzyme activity of 24-hydroxylase. Moreover, we have demonstrated that the magnitude of the increase in enzyme expression is proportional to calcium and vitamin D restriction. Excess of either 1,25(OH)₂D₃ or dexamethasone led to marked stimulation of 24-hydroxylase expression in mouse kidney, while the administration of both together did not additively induce the enzyme, suggesting that substantial amounts of 1,25(OH)₂D₃ result in maximal induction of 24-hydroxylase expression in these mice. In NCD mice with moderate induction of 24-hydroxylase, dexamethasone significantly elevated the enzyme expression. This suggests that 24,25(OH)₂D₃ production is increased in disorders such as multiple sclerosis, which are treated with excess 1,25(OH)₂D₃ or dexamethasone.

Figure 7 Effects of dexamethasone (Dex) on 1,25(OH)₂D₃-induced increase in renal 24-hydroxylase mRNA abundance and enzyme activity in mice. Mice fed the NCD diet were injected s.c. with either vehicle or 1,25(OH)₂D₃ (0.24 nmol/kg) and either vehicle (dotted bars) or 2 mg/kg dexamethasone (solid bars) for 5 successive days. Animals were killed 24 h after the last injection. (A) Total RNA (20 µg) was extracted and subjected to Northern blot analysis. 24-Hydroxylase mRNA concentration was normalized relative to that of cyclophilin mRNA. (B) Renal 24-hydroxylase activity in mice was measured as described in Fig. 6. The activities (means ± S.E.M) of vehicle – Dex, vehicle+Dex, +1,25(OH)₂D₃ – Dex and +1,25(OH)₂D₃+Dex were 43±0.5, 78±6, 437±18, and 561±107 fmol/mg protein per min. Mice treated with both 0.24 nmol 1,25(OH)₂D₃ and 2 mg dexamethasone had maximal activity of 24-hydroxylase which is expressed as 100%. Data are means ± S.E.M. of four mice. **P<0.01 and ***P<0.001 difference between vehicle- and dexamethasone-treated groups. NS, not significantly different.
pharmacologic doses of glucocorticoids. Alterations in vitamin D₃ metabolism may contribute to the loss of bone mineral induced by the steroids.

The present study also showed that dexamethasone did not alter VDR expression in mouse kidneys. Several reasons could help to explain the increased expression of renal 24-hydroxylase seen with glucocorticoid treatment. Since dexamethasone treatment markedly elevated 24-hydroxylase mRNA abundance in UMR-106 osteoblastic cells (I Kurahashi & N Horiuichi, unpublished results), the steroid could act directly on the cells and activate 24-hydroxylase gene transcription via putative glucocorticoid-responsive elements. Although, these elements have not been identified in the 24-hydroxylase promoter. Recent studies (Armbrrecht et al. 1996, 1998) have reported the importance of vitamin D metabolism in bone, especially in UMR-106 osteoblastic cells. PTH and insulin potentiate the stimulation of 24-hydroxylase expression by 1,25(OH)₂D₃. This stimulation could reflect tissue-specific requirements for 1,25(OH)₂D₃ catalysis rather than increase synthesis of 24,25(OH)₂D₃ in bone. Akeno et al. (1997) showed that mice administered a pharmacologic dose of 1,25(OH)₂D₃ express relatively small amounts of the 24-hydroxylase transcripts in bone tissue, suggesting that it is not the predominant site of vitamin D catalysis in vivo. Thus, further studies will be required to define the physiologic and pharmacologic roles of the stimulation of 24-hydroxylase expression by 1,25(OH)₂D₃ and glucocorticoid in bone.

An increase in the metabolic clearance rate of 1,25(OH)₂D₃ results from elevations of renal and intestinal 24-hydroxylase in rats with vitamin D₃ excess (Beckman et al. 1995). However, dexamethasone induced 24-hydroxylase mRNA expression and enzyme activity in the present study without a significant associated change in serum 1,25(OH)₂D₃ in NCD and LCD mice. The most likely explanation for the difference is vitamin D status in animals. NCD and LCD mice treated with dexamethasone had relatively small amounts of 24-hydroxylase expression compared with those in animals with vitamin D₃ or 1,25(OH)₂D₃ excess. Suppression of 1α-hydroxylase activity by the steroids was small (only 30% reduction) in LCD mice and was not observed in NCD mice. Therefore, it is likely that increase in 24-hydroxylase expression by dexamethasone administration does not affect the plasma accumulation of 1,25(OH)₂D₃ in low and normal vitamin D status. This finding is consonant with the results of clinical studies performed previously (Seeman et al. 1980, Prunmell et al. 1991).

In summary, the present study has demonstrated that in LCD mice glucocorticoid excess markedly stimulates renal 25(OH)D₃-24-hydroxylase expression and decreases renal 25(OH)D₃-1α-hydroxylase expression without change in VDR number. Studies are currently in progress to define the details of the signal transduction pathway responsible for 24-hydroxylase gene activation by glucocorticoid.

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