View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Kidney International, Vol. 40 (1991), pp. 212–218

Effects of $1,25(OH)_2D_3$ on compensatory renal growth in the growing rat

STEPHAN MATTHIAS, REINHOLD BUSCH, JÜRGEN MERKE, GERHARD MALL, MONIQUE THOMASSET, and EBERHARD RITZ

Departments Internal Medicine and Pathology, Ruperto Carola University, Heidelberg, Germany, and INSERM U 120, Hopital Robert Debre, Paris, France

Effects of $1,25(OH)_2D_3$ on compensatory renal growth in the growing rat. Renal compensatory growth after uninephrectomy (UNX) was examined in vitamin D replete male 100 g Sprague-Dawley rats. Five days after UNX, the contralateral kidney wet weight increased by 25% with the kidney weight/body weight ratio reaching a plateau by day 7 after UNX. The early weight increase was primarily due to an increased cell number, as evaluated by a stereological technique in perfusion-fixed kidneys. Twenty pmol $1,25(OH)_2D_3$ by daily s.c. injection increased time-averaged $1,25(OH)_2D_3$ concentrations 3.3-fold and reduced the increment in the kidney weight of UNX pairfed rats compared to solvent UNX controls. The number of mitoses (whole kidney and different nephron segments) were significantly reduced by giving $1,25(OH)_2D_3$ to UNX animals at different levels of food intake. The effect was also demonstrable in PTX animals on a constant infusion of exogenous PTH (100 ng/kg/hr 1,34 bPTH by osmotic minipump). The data suggest that changes of $1,25(OH)_2D_3$ concentration within a physiologically relevant range modulate compensatory (and possibly basal) growth of the kidney.

In a renal carcinoma cell line, Nagakura et al [1] showed that 1-alpha-25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] reduced proliferation of cells in monolayer and suppressed clonogenicity in agar culture. An inhibitory effect of $1,25(OH)_2D_3$ on renal cell proliferation is in keeping with observations in numerous other proliferating cell systems, both malignant [1, 2] and non-malignant [3, 4]. More recently, a biphasic effect of $1,25(OH)_2D_3$ on growth with stimulation at very low and inhibition at higher concentrations has been documented in some in vitro systems [5].

In the rat, renal compensatory growth after uninephrectomy is modulated by calcium, PTH and $1,25(OH)_2D_3$ as shown by Jobin and Bonjour [6]. These authors examined vitamin D-deficient rats and noted stimulation of growth by $1,25(OH)_2D_3$ administered by osmotic minipump. The contrasting inhibitory effect of relatively low concentrations of $1,25(OH)_2D_3$ found by Nagakura et al [1] was observed in renal cells grown in the presence of $1,25(OH)_2D_3$ (which is an obligatory component of fetal calf serum). This consideration led us to the hypothesis that the results in vitamin D depleted animals [6] might reflect the results of supplementation of an essential growth factor and might not permit conclusions with respect to potential regulatory functions of $1,25(OH)_2D_3$ in renal cell proliferation and growth.

To further address this issue, we did the following: (i) examined the effects of $1,25(OH)_2D_3$ on renal weight, zonal architecture, cell number and cell volume after uninephrectomy in growing, male Sprague-Dawley rats under conditions of controlled food intake, and (ii) examined the effect of $1,25(OH)_2D_3$ on cell proliferation in vivo in colchicin pretreated animals.

Methods

Animals

Unless specified otherwise, 100 g male Sprague-Dawley rats (Ivanovas Co., Kisslegg/Allgäu, Germany) were kept in single cages in an environment with a controlled light on (12 hr)/light off (12 hr) cycle and with constant temperature (22°C) and humidity (70%). In some experiments, animals of different weights (230, 370, and 480 g) were used to establish age-dependency of renal compensatory growth. Animals had either free access to food or were fed as specified below. The diet was Altromin C 1000 (Altromin Co., Lage/Lippe, Germany) containing 500 U/kg (13 μg/kg) vitamin D_3, 0.95% Ca and 0.65% Pi (wt/wt).

Protocols

Experiment 1. Effect of $1,25(OH)_2D_3$ on normal renal growth and compensatory renal growth after uninephrectomy (UNX), respectively. Under light ether anaesthesia, rats were either UNX (decapsulation of kidney sparing the adrenal) or sham operated, that is, renal decapsulation (CO). Animals were examined at timed intervals as indicated in the Tables and Figures. At the end of the experiment, animals were weighed, serum chemistries were measured and organ weights were determined (wet and dry weight of kidney, liver, heart, spleen, testis, epididymal fat, and soleus muscle). In all experiments, groups of 6 to 8 animals were studied. The food conversion ratio [body weight gain (g)/food consumption (g)] was calculated as described [7].

Received for publication May 14, 1990
and in revised form March 8, 1991
Accepted for publication March 8, 1991

© 1991 by the International Society of Nephrology

To study the effect of $1,25(OH)_2D_3$, 20 pmol/100 g body wt (or solvent) were administered by once daily s.c. injections for the duration of the experiment, starting on the first day after UNX.

Experiment 2. Histological analysis of the kidney after uninephrectomy. Animals were operated on as described above. On the fifth day after surgery, the viscera were fixed by retrograde vascular perfusion at a pressure of 110 mm Hg after catheterization of the abdominal aorta. Before fixation the vascular system was flushed with a dextran solution (Rheomacrodex[R]) containing 0.5 g/liter procaine HCl for two minutes to improve the microcirculation. The inferior vena cava was incised to drain the blood. The incision was performed 10 seconds after starting the dextran infusion in order to avoid the collapse of capillaries caused by low venous pressures. The vascular system was subsequently perfused with 0.2 M phosphate buffer containing 3% paraformaldehyde for 12 minutes.

Experiment 3. Influence of $1,25(OH)_2D_3$ on mitoses in kidneys of parathyroid intact rats. Animals were operated as described above. On the second day after UNX animals received 5 i.p. injections of colchicin (Demecolcin[R]; Serva Co., Heidelberg, Germany) or solvent at 30-minute intervals prior to the end of the experiment in the forenoon hours (yielding a total colchicin dose of 800 μg). Kidneys were excised under ether anaesthesia and fixed in 5% buffered formalin.

$1,25(OH)_2D_3$ treated animals consumed less food. Since in a pilot experiment in animals on unrestricted food intake $1,25(OH)_2D_3$ treatment had diminished mitoses in proximal tubule cells, we restricted food intake in solvent treated animals; they received 60% of the food consumed on the day before by matched $1,25(OH)_2D_3$ treated animals. Partial food deprivation in controls was chosen to exclude the possibility that reduction of mitoses in $1,25(OH)_2D_3$ treated animals was only the result of lower food intake.

Experiment 4. Influence of $1,25(OH)_2D_3$ on mitoses in kidneys of parathyroidectomized rats maintained on PTH by osmotic minipump. Parathyroids were removed using microsurgical techniques. At the time of surgery 100 ng/kg/hr of 1,34 bPTH (Bissendorf Co., Wedemark, Germany) was administered by s.c. osmotic minipump (Alzet Co., model 2 ML 1; 1,34 bPTH was dissolved in cystein hydrochloride). UNX was performed on the fifth day after PTX. Subsequently, animals were given either solvent or 20 pmol/100 g body wt of $1,25(OH)_2D_3$ by daily s.c. injection. Animals were on ad libitum food intake. After colchicin treatment (experiment 3) the experiment was terminated on the seventh day after PTX. Animals were kept on Altromin C 1000 diet throughout the experiment. Kidneys were prepared for counting of mitoses as described in experiment 3. The same experiment was repeated in animals which were put on high Ca diet after parathyroidectomy to achieve higher serum calcium levels. Animals received Altromin C 1000 diet with 1.6% Ca content (by addition of $CaCO_3/CaHPO_4$ 50/50 wt/wt).

Analytical techniques

Serum chemistry. Serum creatinine, calcium, phosphate and total protein were measured using an Autoanalyzer. $1,25(OH)_2D_3$ was measured after Extralut extraction using a radioimmunoassay [8].

Morphometric techniques. Kidneys were carefully removed and temporarily embedded in agar. A random set of equidistant slices (distance 0.75 mm) was prepared from the whole organ. Orientation of sectioning was approximately normal to the axis between the poles. All slices were embedded in Paraplast. Sections of 4 μm thickness were stained with haematoxylin and eosin. The cortical section area (A_c) and the total section area (A_{tot}) were determined in each section with the semiautomatic image analyzing system IBAS I (Kontron Co., Eching, Germany) at a magnification of 10:1. The ratio A_c/A_{tot} corresponded to the volume density V_V of cortical tissue (cm^3 cortical volume per cm^3 total renal tissue volume) according to Delesse's principle [9]. Cortical volume (CV) was derived from V_V of cortical tissue, weight of kidney and specific weight (1.04 g/cm^3) according to the relation: V_v cortex × organ weight/specific weight. The volume fraction of the proximal tubular cells within the cortex (V_V: volume of cells per unit cortical volume) was determined by means of a point counting procedure [9]. Forty test areas were randomly selected from all sections with sytematic area-weighted sampling. Counting was performed with a Zeiss eyepiece containing 100 test points. The proximal tubular cell volume was calculated by multiplication of the V_V ratio with cortical volume. In addition, the number of nuclear profiles of proximal tubular epithelial cells was counted in each test area (45000 μm^2). The number of cell nuclei per unit cortical volume (N_V) was estimated according to the equation

$$N_V = \frac{n}{A\,(t + D - 2h)}$$

where

n = number of counted section profiles,
A = reference sectional area,
t = section thickness,
D = diameter of spherical objects, and
h = loss of small cap sections (truncation) due to contrast deficiency.

Proximal tubular cell nuclei can be considered as spheres. Measurements on nuclei revealed a diameter of approximately 8 μm; the truncation effect was 2 μm and section thickness was 4 μm. The total number of proximal tubular cell nuclei was calculated by multiplication of N_V with the total cortical volume.

Note that in the case of mononuclear proximal tubular cells the ratio of the total proximal tubular volume/total number of proximal tubular cell nuclei is an estimator of the mean volume of the cells.

Counting of mitoses in renal tissue. In HE stained 8 μ sections of paraffin embedded tissue, mitoses were counted at 400 × magnification using a Zeiss Co. Integrationsplatte II 100/25. Mitoses were counted in 50 fields each in the cortex, outer stripe, inner stripe and inner medulla using a systematic random sampling technique.

Measurements of calbindin-D_{28k} in kidney tissue. UNX animals given solvent or 20 pmol/100 g body wt of $1,25(OH)_2D_3$ by daily s.c. injection were examined five days after surgery. For the determination of CaBP content the kidney was rapidly removed from uninephrectomized animals treated with $1,25(OH)_2D_3$, frozen in liquid nitrogen and stored at −80°C.

The 100,000 g supernatants were prepared according to a previously described technique [10]. CaBP was measured by radioimmunoassay as reported previously [10] with antibodies

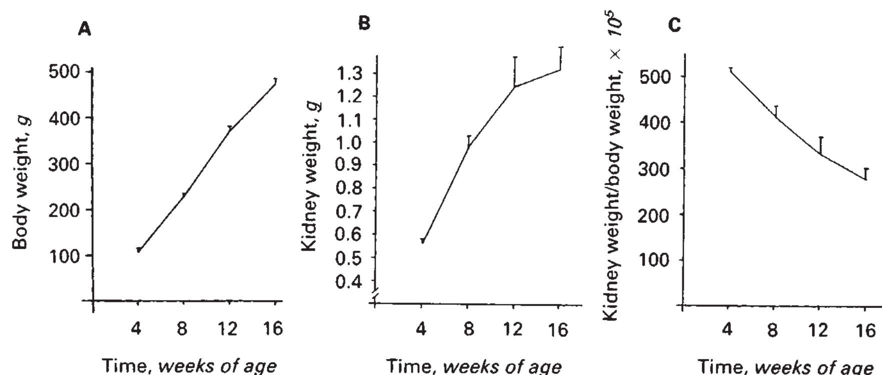

Fig. 1. *Body weight and kidney wet weight as a function of animal age.*

Table 1. Stereological analysis of kidneys five days after uninephrectomy

	Sham operated	P	UNX
Cortical volume *cm³*	0.687 ± 0.0247	<0.02	0.8670 ± .0962
Volume fraction of prox. tubular cells (as fraction of cortical volume)	0.4710 ± .0278	<0.01	0.657 ± 0.0482
Proximal tubular cell number (× 10^6) per kidney	128 ± 23.3	<0.05	170 ± 25.1
Average proximal tubular cell volume μ^3	3672 ± 278	NS	3862 ± 319

Eight animals per group; numbers are means of individual animals.

Table 2. Effect of $1,25(OH)_2D_3$ on compensatory renal growth after uninephrectomy

	Kidney wet weight (*g*)/body weight (*g*) ratio (× 10^5) *days post-operative*		
	3	5	7
UNX solvent	625 ± 38	603 ± 33[a]	626 ± 50[a]
UNX + $1,25(OH)_2D_3$	576 ± 55[b]	515 ± 20[c]	534 ± 36[c]
Sham operation solvent	513 ± 74	472 ± 24	471 ± 12
Sham operation + $1,25(OH)_2D_3$	477 ± 38	428 ± 29	413 ± 31

Eight animals per group; animals ad libitum fed; data as $\bar{x} \pm$ SD.
Difference between UNX and sham op animals [a] $P < 0.01$
Difference between solvent and $1,25(OH)_2D_3$ [b] $P < 0.01$, [c] $P < 0.005$

to rat kidney CaBP prepared in rabbits [11]. CaBP-D_{28k} concentration has been expressed in terms of μg/mg of soluble proteins.

Statistics

Data are given as x ± SD. Differences between groups were examined using Wilcoxon's rank-sum test for random samples.

Results

Renal weight and renal morphology after uninephrectomy

While animal weight increased linearly with age, renal weight failed to do so, and consequently renal (wet) weight/body weight ratio decreased with age (Fig. 1). All subsequent experiments on renal growth were carried out in animals which were four weeks of age. Uninephrectomy caused a significant increase of the renal wet weight/body weight ratio. The ratio reached a plateau seven days after uninephrectomy. Body weight was not altered by UNX nor were serum creatinine, Hb and electrolytes (data not given).

It was the purpose of the study to analyze potential effects of $1,25(OH)_2D_3$ on cell proliferation. To verify whether cell proliferation, that is, hyperplasia, occurred in the rat after UNX, we analyzed (experiment 2) cortical volume and proximal tubular cell number using stereological techniques (Table 1). Five days after UNX, a significant ($P < 0.02$) increase in cortical volume, volume fraction of proximal tubular cells and proximal tubular cell number with no change of average proximal tubular cell volume was noted in UNX rats compared with sham operated, ad libitum-fed control rats. Food consumption was similar in the two groups.

Influence of $1,25(OH)_2D_3$ treatment on renal weight

$1,25(OH)_2D_3$ in non-hypercalcemic doses significantly reduced the renal wet weight/body weight ratio in sham operated solvent versus sham operated, treated ad libitum-fed animals (Table 2).

Subcutaneous injection of 20 pmol $1,25(OH)_2D_3$ raised plasma $1,25(OH)_2D_3$ concentration from 84 ± 7.35 μg/liter at baseline to 350 ± 30.6 (2 hr), 422 ± 12.6 (6 hr) and 108 ± 2.89 (24 hr). The time-averaged $1,25(OH)_2D_3$ concentration (area under the curve) was 3.3-fold higher than baseline concentration.

Table 3 shows that $1,25(OH)_2D_3$ also lowered food consumption and the food conversion ratio, that is, body weight gain per unit weight of consumed food in UNX animals. This resulted in a diminished percent increase in body weight. Renal dry weight and percent increase of renal dry weight were significantly ($P < 0.05$) lower with $1,25(OH)_2D_3$ treatment. When corrected for body weight, the renal wet weight/body weight ratio was still significantly lower (Table 2). The ratio of renal dry weight/body weight (Table 3) was numerically lower but the difference narrowly ($P < 0.06$) failed to reach statistical significance. This result was confirmed in two independent experiments. No difference in absolute weights or the organ weight/body weight ratio was seen for spleen, heart, testis, femur and liver (data not given).

To further evaluate the action of $1,25(OH)_2D_3$ treatment on kidneys, renal content of calbindin-D_{28k} was examined.

Table 3. Effect of $1,25(OH)_2D_3$ on body weight and renal growth after uninephrectomy

	UNX solvent	UNX $1,25(OH)_2D_3$
Final body weight *g*	161 ± 15	148 ± 19
Percent body weight increase	18.4 ± 7.5	15.2 ± 6.5
Renal dry weight *g*	0.232 ± 0.020	0.207 ± 0.022[b]
Percent increase in renal dry weight[a]	59.5 ± 7.5	45.8 ± 10.5[b]
Renal dry weight/body weight ratio (× 10^5)		
Time of surgery	107 ± 3.9	110 ± 6.7
Final	151 ± 21.9	140 ± 6.4
Percent increase	40.9 ± 17.6	26.9 ± 8.1
Food consumption *g/8 days*	146 ± 8.2	132 ± 21.7
Food consumption/body weight ratio	0.2 ± 0.016	0.16 ± 0.037
Serum Ca *mmol/liter*	2.48 ± 0.05	2.77 ± 0.37
Serum alkaline phosphatase *U*	480 ± 47.9	454 ± 94.3

Eight to 10 animals per group; animals ad libitum fed and examined 8 days after uninephrectomy.
[a] Percent increase of remaining kidney (day 8) compared with weight of contralateral kidney (day 0)
[b] Difference solvent vs. $1,25(OH)_2D_3$, $P < 0.05$

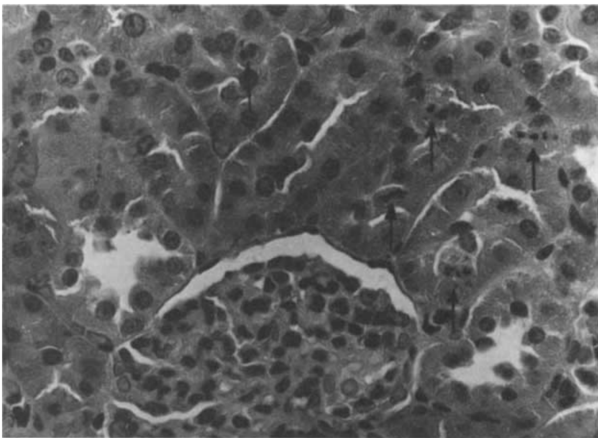

Fig. 2. *Kidney section in colchicin pretreated animals.* Note presence of mitoses (marked by arrows). (HE × 500).

$1,25(OH)_2D_3$ significantly ($P < 0.05$) increased calbindin (ng/mg protein) from 4.39 ± 0.39 to 6.127 ± 0.44. No significant change of serum creatinine (mg/dl) was noted (0.51 ± 0.06 vs. 0.52 ± 0.06).

Influence of $1,25(OH)_2D_3$ treatment on mitoses in kidney

As shown in Figure 2, colchicin pretreatment permitted easy detection of mitoses in HE-stained kidney sections.

Four-week-old animals were examined two days after UNX or sham operation. The results were confirmed in three independent series. In a first series, in animals on unrestricted food intake, $1,25(OH)_2D_3$ treatment reduced food intake (mean 19 ± 2.8 g/2 days vs. 24 ± 3.7 g/2 days in solvent treated controls). Mitoses in total cortex as well as in proximal and distal tubules are shown in Table 4. Very few mitoses were noted in glomeruli. Occasional mitoses were seen in interstitial cells, particularly in the interspace between medullary structures and uroepithelium. Serum calcium levels tended to be higher in $1,25(OH)_2D_3$ treated animals but stayed within the normal range.

To exclude the possibility that the low mitoses seen in $1,25(OH)_2D_3$ treated animals were due to an artifact caused by lower food intake, two further series were done in which solvent-treated UNX animals received only 5 g and 6 g Altromin C diet on day 0 and 1 after uninephrectomy versus 10 g and 12 g in $1,25(OH)_2D_3$ treated animals. The two series gave similar results, and the results of one series are summarized in Figure 3. With $1,25(OH)_2D_3$ treatment, a significant decrease in mitoses was found in the cortex (Fig. 3) as well as in the outer stripe and inner stripe (data not given), despite the fact that solvent treated animals had lost *more* body weight in the two day experimental period (24.7 ± 3.5 g) than did the $1,25(OH)_2D_3$ treated animals (13.1 ± 4.0 g). Low numbers of mitoses and no significant effect of $1,25(OH)_2D_3$ were noted in the inner medulla (21.4 ± 9.3 vs. 19.8 ± 10.5/mm^2) in solvent treated controls. Similarly, mitoses in glomeruli were low and influenced neither by uninephrectomy nor by $1,25(OH)_2D_3$ (day 2: UNX + $1,25(OH)_2D_3$ 10.6 ± 2.2/200 glomeruli vs. 10.1 ± 1.96/200 glomeruli in UNX solvent; day 6 11.4 ± 3.4 in UNX + $1,25(OH)_2D_3$ vs. 11.6 ± 2.45 in UNX solvent). A tendency was noted for lower mitoses to occur in kidneys of $1,25(OH)_2D_3$ treated, sham operated animals, that is, without UNX (data not given).

Influence of $1,25(OH)_2D_3$ treatment on mitoses in kidneys of parathyroidectomized rats maintained on PTH by osmotic minipump

In experiment 4 the chosen dose of PTH failed to fully correct hypocalcemia in PTX animals both on normal and on high calcium diet, but food intake and the increase of body weight were not different from solvent controls (data not shown). Appropriate function of minipumps was verified by inspection at the end of the experiment.

Administration of $1,25(OH)_2D_3$ caused slightly higher serum calcium levels, but failed to correct the hypocalcemia. A significant reduction of mitoses in animals on $1,25(OH)_2D_3$ was noted in the cortex, proximal tubules and distal tubules.

A significant correlation between serum calcium and mitoses was not found in any of the groups. As shown in Table 4, lower mitoses in $1,25(OH)_2D_3$ treated animals were associated with higher serum calcium levels in PTX animals. No significant increase of serum calcium was noted in $1,25(OH)_2D_3$ treated PT-intact animals, but despite this the number of mitoses was lower.

Discussion

The present study provides evidence that $1,25(OH)_2D_3$ interferes with compensatory growth of the residual kidney after uninephrectomy in young growing rats. This was demonstrated by reduction of the number of mitoses in proximal tubules and distal nephron segments. In addition, $1,25(OH)_2D_3$ treated animals had significantly less gain in renal wet weight, in the renal wet weight/body weight ratio and in renal dry weight; the increment of renal dry weight/body weight ratio was numerically lower but narrowly ($P < 0.06$) failed to reach statistical significance.

Table 4. Effect of $1,25(OH)_2D_3$ treatment on mitoses in renal tissue after uninephrectomy: Comparison of PT-intact animals and PTX animals (PTH-infusion by osmotic minipump)

	PT intact normal Ca diet		PTX Normal Ca diet		PTX High Ca diet	
	Solvent	$1,25(OH)_2D_3$	Solvent	$1,25(OH)_2D_3$	Solvent	$1,25(OH)_2D_3$
Final body weight *g*	122 ± 11.3	116 ± 9.4	171 ± 5.2	173 ± 13.6	162 ± 21.6	172 ± 29.4
Serum Ca *mmol/liter*	2.02 ± 0.16	2.22 ± 0.18	1.14 ± 0.38	1.51 ± 0.25	1.49 ± 0.08	1.76 ± 0.18[a]
Mitoses *per mm² cortical area*						
Cortex total	329 ± 39.5	192 ± 34.0[b]	354 ± 185	152 ± 32.2[a]	152 ± 36.2	95 ± 14.2[b]
Proximal tubule	254 ± 26.8	138 ± 19.8[b]	286 ± 131	122 ± 34.4[a]	114 ± 24.7	74.1 ± 11.5[b]
Distal tubule	71.8 ± 19.8	51.7 ± 16.8[b]	68 ± 30.7	30 ± 6.3[a]	37.5 ± 12.2	21.1 ± 3.5[b]

Group size 7 to 10 animals, animals fed ad libitum.
Difference solvent vs $1,25(OH)_2D_3$ [a] $P < 0.05$, [b] $P < 0.005$

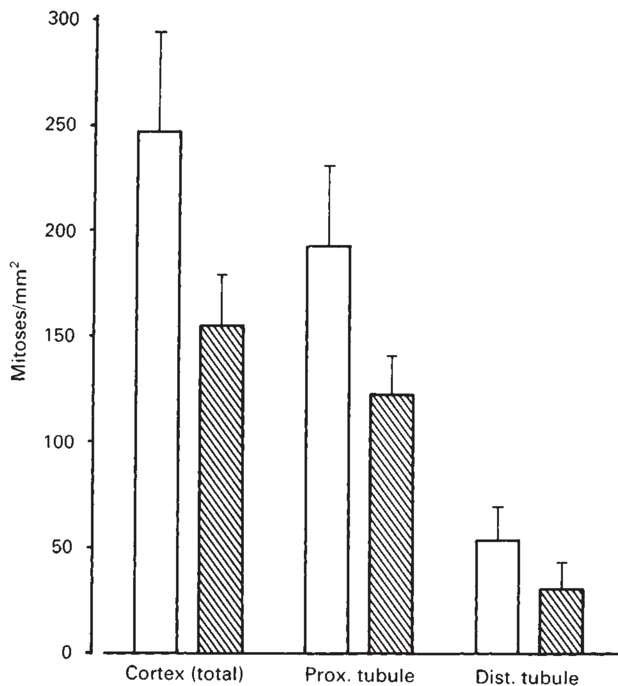

Fig. 3. *Effect of $1,25(OH)_2D_3$ treatment on mitoses in kidneys after uninephrectomy.* Symbols are: (□) solvent; (▧) $1,25(OH)_2D_3$. Mitoses is per mm² cortical area. There were nine animals per group; animals were with restricted food intake. Animals were studied 2 days after uninephrectomy. * Significant difference ($P < 0.005$) between solvent and $1,25(OH)_2D_3$.

Several potential artifacts were considered. Food intake is an important modulator of mitotic activity in renal tissue [12]. Nonhypercalcemic doses of $1,25(OH)_2D_3$ reduced both food intake and the food conversion ratio [7]. This phenomenon was reproducible in several independent series. To exclude an artifact of lower food intake we compared $1,25(OH)_2D_3$ treated, ad libitum-fed animals with solvent treated controls which had received less food. The difference in mitoses remained highly significant. This observation clearly dissociates the effect of $1,25(OH)_2D_3$ treatment and low food intake, respectively.

In parathyroid intact animals it is impossible to distinguish direct effects of $1,25(OH)_2D_3$ and indirect effects mediated via suppression of endogenous PTH. To circumvent this problem we compared solvent treated and $1,25(OH)_2D_3$ treated animals which had undergone PTX and which were maintained on PTH by osmotic minipump. Significant suppression of mitoses in renal tissue by $1,25(OH)_2D_3$ was still noted in animals with constant PTH. This observation excludes the possibility that the effects of $1,25(OH)_2D_3$ are mediated by reciprocal changes of PTH secretion. This point is of importance since Jobin and Bonjour [6] had demonstrated that PTH influences renal growth in an ablation model. It is also of interest that an altered response of adenylate cyclase to PTH has been demonstrated during compensatory renal growth [13].

In PTX animals the effects of $1,25(OH)_2D_3$ were seen despite subnormal serum calcium levels. PTX animals with $1,25(OH)_2D_3$ treatment had lower mitoses; serum calcium concentrations, by necessity, were higher. In PT intact animals, however, $1,25(OH)_2D_3$ caused a similar decrease of mitoses with no significant increase in serum calcium concentration. Therefore it is unlikely that the action of $1,25(OH)_2D_3$ was mediated by changes in serum calcium. In the experiments of Jobin and Bonjour [6] a positive correlation between compensatory renal growth and plasma calcium was noted in animals given PTH or $1,25(OH)_2D_3$, while an inverse correlation was seen in intact animals.

An important consideration is the choice of $1,25(OH)_2D_3$ dosage. A dose of 20 pmol per day was chosen since this dose is slightly higher than the estimated daily rate of production of $1,25(OH)_2D_3$ in the experiments of Hsu et al [14]. Furthermore, in vitamin D-deficient rats, the dose of 20 pmol is slightly above the dose which fully corrects hypocalcemia [6]. Measurements of the time course of serum $1,25(OH)_2D_3$ levels after s.c. injection of $1,25(OH)_2D_3$ showed that serum concentrations averaged over 24 hours (area under the curve) were higher by a factor of approximately 3. It is therefore likely that $1,25(OH)_2D_3$ levels in the present experiment reflect changes within a physiologically relevant range. In addition, measurements of renal calbindin-D_{28K} clearly documented that the chosen dose of $1,25(OH)_2D_3$ acted on target tissues.

It is uncertain whether $1,25(OH)_2D_3$ affects renal growth and renal cell proliferation only when compensatory growth occurs as seen after uninephrectomy. In several independent series a slight, but statistically not significant, reduction of the number of mitoses was also noted in kidneys of sham operated animals. It is possible that the failure to achieve statistical significance is caused by the limited sensitivity of the method used when applied to tissues with low basal mitotic activity. Consequently, an effect of $1,25(OH)_2D_3$ on normal postnatal growth of the

kidney is not excluded. After uninephrectomy, renal growth is selectively stimulated; the gain of renal weight may therefore be particularly susceptible to suppression by $1,25(OH)_2D_3$. In other organs, no effect of $1,25(OH)_2D_3$ on organ weight was noted. This does not necessarily imply that the effect of $1,25(OH)_2D_3$ is restricted to the kidney. To demonstrate an effect of $1,25(OH)_2D_3$ it may be necessary to stimulate growth, for example, of the liver by partial hepatectomy.

One further finding of the present study deserves comment. It has recently been found that hypertrophy is a major mechanism underlying compensatory renal growth in ablation models [15, 16]. This is based on observations in adult animals, where 80% of kidney growth following uninephrectomy is accounted for by hypertrophy predominantly of proximal tubular cells [17, 18]. In mice transient expression of Egr-1, an immediate early gene, has been demonstrated after uninephrectomy [19]. Compensatory renal hypertrophy is characterized by increased kidney ribosome content and increased overall rate of rRNA gene transcription [20, 21]. In this context, inhibition of renal RNA polymerase I and II by high doses of $1,25(OH)_2D_3$ may also be of interest [22].

In our experiments, stereological analysis of kidneys five days after uninephrectomy showed increased numbers of proximal tubular cells with no change of average proximal tubular cell volume, that is, hyperplasia with no detectable hypertrophy. We emphasize that we used young growing rats in which spontaneous renal growth [23] and growth after surgical ablation [24] occurs mainly through hyperplasia. The results in the present study cannot be extrapolated to nongrowing animals.

The finding that $1,25(OH)_2D_3$ interferes with compensatory renal growth in vivo would be in line with previous observations which documented that $1,25(OH)_2D_3$ has antiproliferative actions on numerous cell lines both non-malignant [3] and malignant [1, 2, 25], including renal cell carcinoma lines [1]. Renal tissue binds $1,25(OH)_2D_3$ as shown by autoradiographic studies of Stumpf et al [26], and is further documented by binding studies [27] or by immunohistochemical demonstration of the $1,25(OH)_2D_3$ receptor molecule [28]. $1,25(OH)_2D_3$ receptors are crucial to mediating the effect of vitamin D on target organs. Furthermore, previous authors speculated that expression of $1,25(OH)_2D_3$ receptors in the kidney may change during compensatory renal growth [6]. This prompted us to measure specific binding of ^3H $1,25(OH)_2D_3$ in chromatin fractions of renal tissue (data not given). No major change in specific binding was noted after uninephrectomy, but the biological relevance of this finding is unknown given the changes of cellular composition of the kidneys following uninephrectomy.

In the present study, reduction of mitoses in tubules (but not glomeruli and medullary structures) and diminution of renal weight gain was noted in uninephrectomized animals following treatment with $1,25(OH)_2D_3$. This is in apparent conflict with previous findings of Jobin and Bonjour [6], who noted significant stimulation of compensatory renal growth, assessed by kidney weight, RNA and DNA gains and ^3H-thymidine incorporation, following administration of $1,25(OH)_2D_3$ to vitamin D-depleted Wistar rats. The two studies differ in several respects. We used faster growing Sprague-Dawley rats and normalized renal (dry) weight for body weight gain [which is influenced by $1,25(OH)_2D_3$]. The most salient difference, however, is our use of vitamin D-repleted animals. This may be important since we have recently been able to show [5] that in chondrocytes, low concentrations of $1,25(OH)_2D_3$ stimulated growth and ^3H thymidine incorporation in the presence of charcoal-stripped FCS, that is, in the absence of vitamin D metabolites. In contrast, higher concentrations dose-dependently inhibited chondrocyte proliferation [5]. This finding implies opposite effects of $1,25(OH)_2D_3$ on cell proliferation depending on baseline $1,25(OH)_2D_3$ concentration and $1,25(OH)_2D_3$ dose.

The physiological importance of presumed modulation of renal cell proliferation during compensatory renal growth by $1,25(OH)_2D_3$ is unknown. It is intriguing, however, to speculate that $1,25(OH)_2D_3$ which is synthesized in proximal tubular cells modulates renal cell growth by autocrine and/or paracrine actions. One could also hypothesize that the transient fall in circulating $1,25(OH)_2D_3$ levels as noted by some investigators in patients after uninephrectomy [29, 30] favors compensatory growth of the kidney by disinhibiting cells from the constraining effects of $1,25(OH)_2D_3$. Whether low circulating $1,25(OH)_2D_3$ levels in renal failure favor compensatory growth of residual nephrons (and whether conversely administration of $1,25(OH)_2D_3$ interferes with this process) deserves further study.

Acknowledgments

This study was supported by the Deutsche Forschungsgemeinschaft (Me 632/3-3). The data have been reported in preliminary form at the annual meeting of the American Society of Nephrology in 1988, which was published in *Kidney International* 35:384, 1989. We thank Professor H. Lohrbacher-de Ruiz for continuous help in providing facilities for animal experiments, Ms. U. Hügel for help in $1,25(OH)_2D_3$ receptor preparation, Ms. G. Gorsberg for help in preparing histological sections and Ms. U. Hihler for typing the manuscript.

Reprint requests to Professor Dr. Eberhard Ritz, Department of Internal Medicine, University of Heidelberg, Bergheimer Strasse 58, D-6900 Heidelberg, Germany.

References

1. Nagakura K, Abe E, Studa T, Hayakawa M, Nakamura H, Tazaki H: Inhibitory effect of 1,25-dihydroxyvitamin D_3 on the growth of the renal carcinoma cell line. *Kidney Int* 29:834–840, 1986
2. Eisman JA, Koga M, Sutherland RL, Barkla DM, Tutton PJM: 1,25-dihydroxyvitamin D_3 and the regulation of human cancer cell replication. *Proc Soc Exp Biol Med* 191:221–226, 1989
3. Merke J, Schwittay P, Fürstenberger G, Gross M, Marka S, Ritz E: Demonstration and characterization of 1,25-dihydroxyvitamin D_3 receptors in basal cells of epidermis of neonatal and adult mice. *Calcif Tissue Int* 37:257–267, 1985
4. Hosomi J, Abe E, Suda T, Kuroki T: Regulation of terminal differentiation of cultured mouse epidermal cells by 1,25-dihydroxyvitamin D_3. *Endocrinology* 113:1950–1957, 1983
5. Klaus G, Merke J, Eing H, Hügel U, Milde P, Reichel H, Ritz E, Mehls O: $1,25(OH)_2D_3$ receptor regulation and $1,25(OH)_2D_3$ effects in primary cultures of growth cartilage of the rat. *Calcif Tiss Int* (in press)
6. Jobin JR, Bonjour JP: Compensatory renal growth: Modulation by calcium PTH and 1,25-dihydroxyvitamin D_3. *Kidney Int* 29: 1124–1130, 1986
7. Mehls O, Ritz E, Hunziker EB, Egli P, Heinrich U, Zapf J: Improvement of growth and food utilization by human recombinant growth hormone in uremia. *Kidney Int* 33:45–52, 1988
8. Bouillon R, De Moor P, Baggiolini EG, Uskokovic MR: A radioimmunoassay for 1,25-dihydroxycholecalciferol. *Clin Chem* 26:562–567, 1980
9. Weibel ER: *Sterological Methods*. New York, Academic Press, 1979

10. THOMASSET M, PARKES CO, CUISINIER-GLEIZES P: Rat calcium binding proteins: Distribution, development and vitamin D dependence. *Am J Physiol* 243:E483–E488, 1982
11. INTRATORS S, ELION J, THOMASSET M, BREHIER A: Purification, immunological and biochemical characterization of rat 28 KDa cholecalcin (cholecalciferol-induced calcium binding proteins). *Biochem J* 231:89–95, 1985
12. JACOBSSON B, BOHMAN SO, SUNDELIN B, APERIA A: Mitotic response to high protein intake in different renal cell types in weanling rats. *Kidney Int* 33:662–666, 1988
13. MILANES CL, PERNALETE N, STAROSTA R, PEREZ-GONZALES M, PAZ-MARTINEZ V, BELLORIN-FONT E: Altered response of adenylate cyclase to parathyroid hormone during compensatory renal growth. *Kidney Int* 36:802–809, 1989
14. HSU CH, PATEL S, YOUNG E, SIMPSON RU: Production and metabolic clearance of calcitriol in acute renal failure. *Kidney Int* 33:530–535, 1988
15. FINE L: The biology of renal hypertrophy. *Kidney Int* 29:619–634, 1986
16. HALLIBURTON IW, THOMSON RY: Chemical aspects of compensatory renal hypertrophy. *Cancer Res* 25:1882–1887, 1965
17. JOHNSON HA, VERA ROMA JM: Compensatory renal enlargement: Hypertrophy vs. hyperplasia. *Am J Pathol* 49:1–13, 1966
18. JOHNSON HA: Cytoplasmatic response to overwork, in *Compensatory Renal Hypertrophy*, edited by NOWINSKY WW, GROSS RJ, New York and London, Academic Press, 1969, pp 9–25
19. QUELLETTE AJ, MALT RA, SUKHATME VP, BONVENTRE JV: Expression of two "immediate early" genes, Egr-1 and c-fos, in response to renal ischemia and during compensatory renal hypertrophy in mice. *J Clin Invest* 85:766–771, 1990
20. MALT RA, LEMAITRE DA: Accretion and turnover of RNA in the renoprival kidney. *Am J Physiol* 214:1041–1047, 1968
21. QUELLETTE AJ, MOONKA R, ZELENETZ AD, MALT RA: Regulation of ribosome synthesis during compensatory renal hypertrophy. *Am J Physiol* 253:C506–C513, 1987
22. COLSTON KW, EVANS IMA, SPELSBERG TC, MACINTYRE I: Feedback regulation of vitamin D metabolism by 1,25-dihydroxycholecalciferol. *Biochem J* 164:83–89, 1977
23. WESSON LG: Compensatory growth and other growth responses of the kidney. *Nephron* 51:149–184, 1989
24. HEINE WD, STÖCKER E: Regeneration of kidney parenchyma under normal and pathological conditions. *Beitr Pathol* 145:98–99, 1972
25. EISMAN JA, KOGA M, SUTHERLAND RL, BARKLA DM, TRUTTON PJM: 1,25-dihydroxyvitamin D_3 and the regulation of human cancer cell replication. *Proc Soc Exp Biol Med* 191:221–226, 1989
26. STUMPF WE, CLARK SA, SAR M, REID FA, TANAKA Y, DELUCA HE: Target cells for 1,25-dihydroxyvitamin D_3 in intestinal tract, stomach, kidney, skin, pituitary and parathyroid. *Science* 206: 1188–1190, 1979
27. CHRISTAKOS S, NORMAN AW: Studies on the mode of action of calciferol XXIX. Biochemical characterization of 1,25-dihydroxyvitamin D_3 receptors in chick pancreas and kidney cytosol. *Endocrinol* 108:140–149, 1981
28. CLEMENS TL, GARRETT KP, ZHOU XY, PIKE JW, HAUSSLER MR, DEMPTER DW: Immunocytochemical localization of the 1,25-dihydroxyvitamin D_3 receptor in target cells. *Endocrinol* 122:1224–1230, 1988
29. FRIEDLANDER MA, LEMKE JH, HORST RL: The effect of uninephrectomy on mineral metabolism in normal human kidney donors. *Am J Kidney Dis* 11:393–401, 1988
30. LUCAS PA, BROWN RC, WOODHEAD JS: Acute response of parathyroid hormone and 1,25-hydroxyvitamin D_3 to unilateral nephrectomy in healthy donors. *Nephrol Dial Transplant* 1:199–203, 1986