Suppressive Effects of 24,25-Dihydroxycholecalciferol on Bone Resorption Induced by Acute Bilateral Nephrectomy in Rats

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ABSTRACT The possible suppressive effects of 24,25-dihydroxycholecalciferol on secondary hyperparathyroidism and increased bone resorption were investigated in adult rats raised on a diet normal in calcium, phosphorus, and vitamin D, and subjected to acute bilateral nephrectomy. The animals had received subcutaneous radiocalcium 4 wk before the experiment. 5 h after nephrectomy an increase in serum total calcium, ^{45}Ca-specific activity, citrate, phosphorus, and magnesium concentrations were observed. Serum immunoreactive parathyroid hormone increased, while serum calcitonin decreased. The osteoclast count in the tibial metaphyses was augmented. The biochemical and histological changes observed were partly parathyroid hormone and calcitonin independent, as they also occurred in parathyroidectomized hypocalcemic rats. Pretreatment with 650 pmol of 24,25-dihydroxycholecalciferol 16 h before nephrectomy prevented bone calcium mobilization and diminished the rise in serum total calcium and citrate both in parathyroid-intact and in parathyroidectomized animals. In parathyroid-intact rats, serum immunoreactive parathyroid hormone and calcitonin remained normal in spite of the fall in serum-ionized calcium, and the number of osteoclasts did not increase. In parathyroidectomized rats, 24,25-dihydroxycholecalciferol did not prevent the postnephrectomy rise in the osteoclast count. This latter observation suggests that this metabolite exerts its effect on bone either by acting on cells other than osteoclasts, i.e., the osteocytes, or by inhibiting cell activity. At equimolar dosage 1,25-dihydroxycholecalciferol had a potent stimulatory effect on bone resorption. This effect of 1,25-dihydroxycholecalciferol was partly blocked by the simultaneous administration of 24,25-dihydroxycholecalciferol.

The potential clinical significance of these observations remains to be determined.

INTRODUCTION

It is well established that vitamin D_3 (cholecalciferol) must undergo metabolic conversion in order to promise its physiological actions (1–4). The first conversion occurs in the liver and leads to the formation of 25-hydroxycholecalciferol. This metabolite is the major circulating form of vitamin D_3, and is the precursor of dihydroxylated derivatives: 1,25-dihydroxycholecalciferol (1,25-$[OH]_2D_3$),[1] 24,25-dihydroxycholecalciferol (24,25-$[OH]_2D_3$), and 25,26-dihydroxycholecalciferol (25,26-$[OH]_2D_3$). 1,25-$(OH)_2D_3$ is considered the active, hormonal, form of cholecalciferol because it exerts the

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[1] *Abbreviations used in this paper*: iCT, immunoreactive calcitonin; iPTH, immunoreactive parathyroid hormone; 1,25-$(OH)_2D_3$, 1,25-dihydroxycholecalciferol; 24,25-$(OH)_2D_3$, 24,25-dihydroxycholecalciferol.

two major effects of vitamin D_3, i.e., stimulation of bone resorption and enhancement of intestinal calcium and phosphorus absorption (1–4). The physiological significance of 24,25-$(OH)_2D_3$ and 25,26-$(OH)_2D_3$ is less clear. It has been demonstrated that these two metabolites are considerably less potent than 1,25-$(OH)_2D_3$ on bone calcium mobilization or on intestinal calcium absorption (1, 5, 6). Recent data, however, suggest that 24,25-$(OH)_2D_3$ may play biologically important roles. It has been shown that 1,25-$(OH)_2D_3$ cannot satisfy all of the functions of vitamin D in the laying hen (7), and that 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ are required for the normal development of chick embryos (8). Ornoy et al. (9) have demonstrated that 24,25-$(OH)_2D_3$ alone is sufficient to prevent rachitic bone lesions. Other investigators (10) have shown that 24,25-$(OH)_2D_3$ is able to suppress formation of endosteal fibrosis, even in the absence of 1,25-$(OH)_2D_3$. A suppressive effect of this metabolite on parathyroid gland secretion has been demonstrated in experiments of various design (11–13). In vitro studies from our laboratory produced evidence that 24,25-$(OH)_2D_3$ at very low concentrations stimulates the synthesis of specific proteoglycans by chondrocytes (14) and suppresses the action of agents known to stimulate bone resorption, such as parathyroid hormone, vitamin D, and heparin (15, 16). A few clinical trials have led to the assumption that 24,25-$(OH)_2D_3$ may be the metabolite that principally promotes bone formation (17). At the present time, no information is available on a possible in vivo suppressive effect of this metabolite on bone resorption.

We have investigated this point in view of the potential therapeutic value of 24,25-$(OH)_2D_3$ in the management of the pathological increases in bone resorption. Bilaterally nephrectomized rats were chosen as experimental models, as this procedure provokes a marked increase in bone calcium mobilization. Our results demonstrate that a single pharmacological dose of 24,25-$(OH)_2D_3$, i.e., 650 pmol, has a potent inhibitory effect on the biochemical and histological changes of increased bone resorption observed 5 h after nephrectomy, and partly blocks the stimulatory effect of 1,25-$(OH)_2D_3$ on bone resorption. These effects appear to be related not only to an inhibitory action on parathyroid gland hypersecretion, but also to the depression of bone-cell response to parathyroid hormone, 1,25-$(OH)_2D_3$, and acute uremia.

METHODS

Male Wistar rats weighing 180–200 g were fed a diet containing 0.45% calcium, 0.30% phosphorus, and 30 IU/g of vitamin D_3 (Laboratoire Roussel, Paris, France). The animals were randomly separated into two main groups: those to undergo a bilateral nephrectomy, and those to undergo a sham operation. In each major group a subgroup of animals was injected intraperitoneally with 650 pmol of 24,25-$(OH)_2D_3$ or 1,25-$(OH)_2D_3$, or with a combination of 650 pmol of 24,25-$(OH)_2D_3$ and 650 pmol of 1,25-$(OH)_2D_3$ dissolved in 0.05 ml of 95% ethanol. Control animals were given the solvent alone. Thereafter, all animals were fasted.

16 h after intraperitoneal administration of vitamin D metabolite(s) or the solvent, nephrectomy or a sham operation was performed under ether anesthesia, using a posterolateral approach. During nephrectomy special precautions were used to preserve the adrenals. In each subgroup of animals some rats had been injected with 5 μCi of ^{45}Ca in 0.05 μl of saline solution 4 wk before nephrectomy or sham operation. In addition, some animals injected with solvent alone or with 650 pmol of 24,25-$(OH)_2D_3$ or with both metabolites were parathyroidectomized 48 h before nephrectomy. To ascertain that the animals had been successfully parathyroidectomized, blood samples were obtained 48 h after surgery and serum calcium concentrations were determined. Only those rats with serum calcium concentrations <6.5 mg/dl were used. The synthetic 24R,25-$(OH)_2D_3$ and 1,25-$(OH)_2D_3$ used in these studies were gifts from Dr. M. R. Uskovic (Research Laboratories, Hoffmann-La Roche, Inc., Nutley, N. J.).

5 h after nephrectomy or sham operation, blood samples were collected anaerobically from the abdominal aorta in 3-ml vacuum tubes (Vacutainer, Becton-Dickinson & Co., North Canaan, Conn.), and both tibial metaphyses were removed. Blood samples were analyzed for serum total calcium, ^{45}Ca-specific activity, ionized calcium, citrate, phosphorus, magnesium, immunoreactive parathyroid hormone (iPTH), immunoreactive calcitonin (iCT), total protein concentrations, and blood pH. In tibial metaphyses the osteoclasts were counted.

Serum calcium concentration was determined by a semiautomated complexometric technique with the aid of a Titrator (Marius Calcium Titrator, Amsterdam, Netherlands). Serum phosphorus was measured by the method of Fiske and Subbarow (18), adapted to an Auto Analyzer (Technicon Corp., Domont, France). Magnesium was measured by absorption flame spectrophotometry (Perkin-Elmer Corp., Norwalk, Conn., model 305), and serum total proteins with a refractometer (American Optical Corp., Scientific Instrument Div., Buffalo, N. Y.). Determination of serum citrate was performed according to an enzymatic technique (19). The Space-Stat 2 ionized calcium analyzer (Orion-Biomedical Division, Orion Research, Cambridge, England) was used for serum-ionized calcium determinations. Blood pH was measured at 37°C with a capillary glass pH-electrode (Ingold, Zurich, Switzerland). Parathyroid hormone levels were estimated radioimmunologically with [^{131}I]iodo-bovine PTH-(bPTH [-84]) and anti-bPTH serum (chicken 12 M), as previously described (20). The coefficient of variation of the assay is 12%. Calcitonin levels were measured by radioimmunoassay according to the technique of Heynen and Franchimont (21) modified according to the method of Roos and Deftos (22). For concentrations of iCT varying from 100 to 1,000 pg/ml the interassay variation is 5%. Determinations of ^{45}Ca were performed by spotting 0.5 ml of serum in Instagel solution. The radioactivity was counted with a Packard-Tri-Carb liquid scintillation spectrometer (model 3320, Packard Instrument Co., Downers Grove, Ill.), equipped with an external standardization system.

For osteoclast counts, both proximal tibial metaphyses were fixed in a mixture of 2% glutaraldehyde (Ladd Research Industries, Inc., Burlington, Vt.) and 1.2% formaldehyde (Merck, AG, Darmstadt, West Germany), decalcified in 10% ethylene-diamine-tetracetic acid (Prolabo, Paris, France), and embedded in esterwax (BDH Chemicals Ltd., Poole, England). 4-μm-thick transverse sections were stained with toluidine blue (BDH Chemicals Ltd.,) at pH 6.4. Osteoclasts were identified as large multinucleated cells with foamy cytoplasm

on or near bone surfaces. Osteoclasts with a single nucleus were identified by their cytoplasmic appearance and their typical brush border. All osteoclasts were counted at the ossification zone 2,000 μm below the cartilage plate at magnification ×500 over an area of 2.18 mm². The value for each animal represents the means of the cell numbers in three sections in the middle of the metaphyses at 200 μm distance from each other. The fact that the selected area could be considered representative of the whole metaphyseal zone was previously ascertained by counting in two animals the osteoclast numbers on sections taken at 200-μm distance over the whole metaphysis.

Results were expressed as mean values±SEM. The significance of the differences between groups was assessed by Student's *t* test (23).

RESULTS

Effects of nephrectomy in parathyroid-intact and parathyroidectomized animals. Compared with sham-operated animals, nephrectomized parathyroid-intact rats had significantly higher serum total calcium, citrate, phosphorus, and magnesium concentrations (Table I). Serum ^{45}Ca-specific activity was increased, and ionized calcium concentration significantly decreased 5 h after nephrectomy without any significant change in blood pH or serum total protein concentration. Serum iPTH levels reached 10.5±2.5 vs. 2.0±0.54 μleq/ml in sham-operated controls, and serum iCT was decreased to less than half the control value. In tibial metaphyses a highly significant ($P < 0.001$) augmentation in osteoclast number was found in nephrectomized rats.

In previously parathyroidectomized animals (Table I) nephrectomy was also followed by an increase in serum total calcium, ^{45}Ca-specific activity, phosphorus, and magnesium. However, the increase in

TABLE I

Summary of Results 5 h after Bilateral Nephrectomy or Sham Operation in Parathyroid-intact and Parathyroidectomized Rats

	Parathyroid-intact			Parathyroidectomized		
	Sham-operated	Nephrectomized	*P*	Sham-operated	Nephrectomized	*P*
Serum						
Total calcium, *mg/dl*	10.0±0.1 (20)	13.1±0.1 (28)	<0.001	5.2±0.2 (15)	7.2±0.2 (12)	<0.001
Ionized calcium, *mg/dl*	5.1±0.05 (12)	4.8±0.1 (8)	<0.01	2.7±0.09 (10)	2.5±0.1 (9)	NS
^{45}Ca-specific activity, *cpm × 10³/mg total Ca*	3.5±0.2 (7)	4.4±0.2 (14)	<0.05	2.9±0.03 (7)	3.3±0.1 (14)	<0.001
Citrate, *mg/dl*	3.8±0.2 (12)	26.4±0.9 (15)	<0.001	3.7±0.3 (11)	14.4±0.6 (12)	<0.001
Phosphate, *mg/dl*	8.1±0.2 (18)	13.6±0.4 (20)	<0.001	11.3±0.3 (10)	17.2±0.8 (12)	<0.001
Magnesium, *mg/dl*	2.0±0.07 (12)	3.0±0.1 (15)	<0.001	1.6±0.05 (11)	2.3±0.1 (12)	<0.001
Total protein, *g/dl*	5.7±0.09 (14)	5.7±0.01 (8)	NS	5.9±0.9 (10)	5.7±0.7 (12)	NS
iPTH, *μleq/ml*	2.0±0.54 (10)	10.5±2.6 (10)	<0.01			
iCT, *pg/ml*	260±7 (8)	100±6 (8)	<0.001			
Blood						
pH	7.36±0.03 (12)	7.28±0.03 (9)	NS	7.35±0.01 (10)	7.37±0.02 (9)	NS
Tibial metaphyses						
Osteoclast count, *per 2.18 mm²*	81.1±1.2 (12)	103.5±2.7 (10)	<0.001	56±2.5 (14)	76±2.2 (9)	<0.001

All values are mean±SEM. Numbers in parentheses are the numbers of animals. *P* values compare nephrectomized rats with sham-operated animals in parathyroid-intact and parathyroidectomized groups. NS, not significant.

serum calcium and citrate from control levels was smaller (ΔCa + 2.0 mg/dl and Δcitrate + 10.7 mg/dl) than those observed in parathyroid-intact animals (ΔCa + 3.1 mg/dl and Δcitrate + 22.6 mg/dl). No significant changes were noted in serum-ionized calcium and total protein concentrations, nor were there any changes in blood pH in rats that were parathyroidectomized and nephrectomized. These animals had a decreased number of osteoclasts in their tibial metaphyses, compared with parathyroid-intact rats (56±2.5 vs. 81.1±1.2). The increase in the osteoclast number observed after bilateral nephrectomy was, however, comparable to that noted in parathyroid-intact animals.

Effect of 24,25-$(OH)_2D_3$ pretreatment. In a preliminary study, the effects of pretreatment with 130, 325, and 650 pmol of 24,25-$(OH)_2D_3$ on serum calcium concentration in nephrectomized and sham-operated controls were analyzed. These doses did not induce any significant change in serum calcium concentration of sham-operated controls (10.2±0.2, 10.1±1, and 10.2±0.2 mg/dl in pretreated groups vs. 10.1±0.1 mg/dl in sham-operated controls). They did, however, significantly decrease the hypercalcemic response to nephrectomy (serum calcium concentration: 12.3±0.2, 12.4±0.2, and 11.1±0.2 mg/dl in rats pretreated with, respectively, 130, 325, and 650 pmol of 24,25-$(OH)_2D_3$ vs. 13.1±0.2 mg/dl in untreated controls ($P < 0.05$ and $P < 0.001$). Since 650 pmol of 24,25-$(OH)_2D_3$ showed the greatest activity, this dosage was chosen for further investigation of the effects of this metabolite on biochemical and histological changes induced by nephrectomy.

The intraperitoneal administration of 650 pmol of 24,25-$(OH)_2D_3$ 16 h before nephrectomy significantly reduced the hypercalcemic and hypercitratemic responses to nephrectomy both in parathyroid-intact (Table II) and in parathyroidectomized rats (Table III). These suppressive effects were accompanied by an in-

TABLE II

Summary of Results 5 h after Bilateral Nephrectomy in Parathyroid-intact Rats Pretreated 16 h before Nephrectomy with 24,25-$(OH)_2D_3$. 1,25-$(OH)_2D_3$, 24,25-$(OH)_2D_3$ plus 1,25-$(OH)_2D_3$, or with the Vehicle Alone

	Pretreatment				*P*			
	Vehicle	24,25-$(OH)_2D_3$	1,25-$(OH)_2D_3$	1,25-$(OH)_2D_3$ + 24,25-$(OH)_2D_3$	24,25-$(OH)_2D_3$ vs. vehicle	1,25-$(OH)_2D_3$ vs. vehicle	1,25-$(OH)_2D_3$ + 24,25-$(OH)_2D_3$ vs. vehicle	1,25-$(OH)_2D_3$ + 24,25-$(OH)_2D_3$ vs. 1,25-$(OH)_2D_3$
Serum								
Total calcium, *mg/dl*	13.1±0.1 (28)	11.1±0.2 (22)	14.6±0.3 (10)	12.9±0.6 (10)	<0.001	<0.001	NS	<0.02
Ionized calcium, *mg/dl*	4.8±0.1 (14)	4.8±0.1 (7)	5.4±0.06 (9)	4.9±0.04 (9)	NS	<0.001	NS	<0.001
^{45}Ca-specific activity, *cpm × 10^3/mg total Ca*	4.4±0.2 (14)	3.3±0.2 (6)	5.2±0.1 (9)	4.6±0.1 (10)	<0.01	<0.01	NS	<0.01
Citrate, *mg/dl*	26.4±0.9 (15)	18.1±2.8 (12)	38.8±1.5 (10)	34.3±4.1 (10)	<0.01	<0.001	<0.02	NS
Phosphate, *mg/dl*	13.6±0.4 (10)	13.2±0.1 (21)	10.6±0.3 (20)	12.6±0.4 (9)	NS	<0.01	NS	<0.001
Magnesium, *mg/dl*	3.0±0.1 (15)	2.8±0.1 (11)	3.1±0.1 (10)	5.6±0.04 (9)	NS	NS	NS	NS
Total protein, *g/dl*	5.7±0.01 (8)	5.8±0.06 (13)	5.7±0.1 (10)	5.6±0.04 (8)	NS	NS	NS	NS
iPTH, *μleq/ml*	8.73±0.9 (22)	2.8±0.7 (6)	2.14±0.29 (6)	4.74±0.54 (8)	<0.01	<0.001	<0.02	<0.01
iCT, *pg/ml*	100±6 (8)	265±5 (8)	240±30 (7)	280±26 (10)	<0.001	<0.01	<0.001	NS
Blood								
pH	7.28±0.03 (9)	7.31±0.03 (7)	7.19±0.01 (9)	7.48±0.02 (9)	NS	<0.001	NS	NS
Tibial metaphyses								
Osteoclast count, *per 2.18 mm^2*	103.5±2.7 (10)	86.1±2.8 (8)	95.7±5 (10)	94.9±3.27 (6)	<0.001	NS	NS	NS

All values are mean±SEM. Numbers in parentheses are the numbers of animals. NS, not significant. The dose used for each metabolite is 650 pmol.

TABLE III

Summary of Results 5 h after Bilateral Nephrectomy in Parathyroidectomized Rats Pretreated 16 h before Nephrectomy with 24,25-$(OH)_2D_3$, 1,25-$(OH)_2D_3$, 24,25-$(OH)_2D_3$ plus 1,25-$(OH)_2D_3$, or with Vehicle Alone

	Pretreatment				*P*			
	Vehicle	24,25-$(OH)_2D_3$	1,25-$(OH)_2D_3$	1,25-$(OH)_2D_3$ + 24,25-$(OH)_2D_3$	24,25-$(OH)_2D_3$ vs. vehicle	1,25-$(OH)_2D_3$ vs. vehicle	1,25-$(OH)_2D_3$ + 24,25-$(OH)_2D_3$ vs. vehicle	1,25-$(OH)_2D_3$ + 24,25-$(OH)_2D_3$ vs. 1,25-$(OH)_2D_3$
Serum								
Total calcium, *mg/dl*	7.2±0.2 (28)	5.9±0.3 (20)	12.5±0.3 (10)	9.3±0.4 (17)	<0.001	<0.001	<0.001	<0.001
Ionized calcium, *mg/dl*	2.5±0.1 (14)	2.4±0.2 (9)	4.7±0.1 (10)	4.5±0.1 (7)	NS	<0.001	<0.001	NS
^{45}Ca-specific activity, *cpm × 10^3/mg total Ca*	3.3±0.1 (14)	2.7±0.1 (8)	3.8±0.1 (8)	3.7±0.09 (10)	<0.02	<0.01	<0.05	NS
Citrate, *mg/dl*	14.4±0.6 (12)	12.7±1.2 (7)	23.7±0.2 (10)	15.8±3.1 (7)	NS	<0.001	NS	<0.001
Phosphate, *mg/dl*	17.2±0.8 (12)	15.2±0.3 (19)	14.9±0.7 (6)	14.4±0.4 (8)	NS	NS	<0.01	NS
Magnesium, *mg/dl*	2.3±0.8 (12)	1.9±0.4 (7)	2.9±0.1 (6)	2.51±0.14 (8)	NS	<0.01	NS	<0.05
Total protein, *g/dl*	5.7±0.7 (12)	5.9±0.6 (8)	5.8±0.2 (6)	5.6±0.04 (8)	NS	NS	NS	NS
Blood								
pH	7.37±0.02 (9)	7.38±0.03 (7)	7.36±0.02 (6)	7.46±0.01 (8)	NS	NS	NS	NS
Tibial metaphyses								
Osteoclast count, *per 2.18 mm²*	76±2.2 (15)	72.3±4.4 (7)	91.5±2.7 (6)	102.97±5.12 (7)	NS	<0.01	<0.001	<0.05

All values are mean±SEM. Numbers in parentheses are the numbers of animals. NS, not significant. The dose used for each metabolite is 650 pmol.

hibition of the rise in serum ^{45}Ca-specific activity. In addition, pretreatment with 24,25-$(OH)_2D_3$ completely abolished the postnephrectomy increase in serum iPTH concentration, and prevented the postnephrectomy fall in serum iCT concentration. The increase in metaphyseal osteoclast number was not observed in nephrectomized rats pretreated with 24,25-$(OH)_2D_3$ when the animals were parathyroid intact. In contrast, the metabolite did not prevent the augmentation in the number of osteoclasts in parathyroidectomized rats. The rise in serum phosphorus and magnesium concentrations and the fall in serum ionized calcium concentration were comparable in 24,25-$(OH)_2D_3$ or vehicle-pretreated animals.

Effect of 1,25-$(OH)_2D_3$ pretreatment. To compare the effect of 24,25-$(OH)_2D_3$ and 1,25-$(OH)_2D_3$ in this experimental model, 650 pmol of 1,25-$(OH)_2D_3$ were injected 16 h before nephrectomy or sham operation in parathyroid-intact and parathyroidectomized rats (Table III). Pretreatment with 1,25-$(OH)_2D_3$ had opposite effects when compared with pretreatment with 24,25-$(OH)_2D_3$: the degree of hypercalcemia was more pronounced, whereas the serum-ionized calcium, ^{45}Ca-specific activity, citrate, and magnesium concentrations were further increased in parathyroid-intact and parathyroidectomized rats. The administration of 1,25-$(OH)_2D_3$ significantly decreased serum phosphate in parathyroid-intact nephrectomized rats. Such a decrease in high serum phosphate after 1,25-$(OH)_2D_3$ administration has been reported (24). Serum iPTH and iCT levels remained unchanged after nephrectomy. The osteoclast number in tibial metaphyses was not significantly changed by the administration of 1,25-$(OH)_2D_3$ in parathyroid-intact rats, but markedly increased in parathyroidectomized rats. Compared with 24,25-$(OH)_2D_3$ or vehicle-pretreated animals, 1,25-$(OH)_2D_3$-pretreated rats had lower blood pH after nephrectomy. As with 24,25-$(OH)_2D_3$ treatment, 1,25-$(OH)_2D_3$ administration did not influence the serum total protein concentration.

Effect of 1,25-$(OH)_2D_3$ plus 24,25-$(OH)_2D_3$ pretreatment. Given the fact that two dihydroxylated metabolites of vitamin D exhibited different effects, it was of interest to see whether 24,25-$(OH)_2D_3$ could inhibit the bone resorbing action of 1,25-$(OH)_2D_3$. For this purpose, both metabolites were injected simul-

taneously 16 h before nephrectomy or sham operation to parathyroid-intact and parathyroidectomized rats. Results show that in parathyroid-intact animals (Table II) the administration of 24,25-$(OH)_2D_3$ attenuates the hypercalcemic effect of 1,25-$(OH)_2D_3$. The serum-specific activity of ^{45}Ca was also decreased in rats pretreated with 24,25-$(OH)_2D_3$ plus 1,25-$(OH)_2D_3$, when compared with those treated with 1,25-$(OH)_2D_3$ alone. However, the increase in osteoclast number and in serum citrate of 1,25-$(OH)_2D_3$-pretreated rats was not affected by the simultaneous administration of 24,25-$(OH)_2D_3$. Pretreatment with 24,25-$(OH)_2D_3$ abolished the decrease in serum phosphate and normalized the blood pH induced by 1,25-$(OH)_2D_3$ administration.

Although in parathyroidectomized rats (Table II) the presence of 24,25-$(OH)_2D_3$ also attenuated the hypercalcemic and hypermagnesemic effects of 1,25-$(OH)_2D_3$,the hypercalcemic effect of 1,25-$(OH)_2D_3$ predominated over the hypocalcemic action of 24,25-$(OH)_2D_3$. The increase in serum-specific activity of ^{45}Ca and in osteoclast number due to 1,25-$(OH)_2D_3$ pretreatment persisted in spite of the simultaneous administration of 24,25-$(OH)_2D_3$. As with 24,25-$(OH_2D_3$ or 1,25-$(OH)_2D_3$, the administration of both metabolites did not influence the serum total protein concentrations.

DISCUSSION

The results of the present investigation indicate two conclusions. The first concerns some biological anomalies manifest 5 h after bilateral nephrectomy in rats, i.e., the increase in bone resorption and the marked rise in serum calcium concentration. The second conclusion concerns the comparative effects of 24,25-$(OH)_2D_3$ and 1,25-$(OH)_2D_3$ in this model of acute renal failure.

The hypercalcemia that characterizes the early period following bilateral nephrectomy is a well-known phenomenon in experimental animals (25–29). It has also been occasionally reported in man suffering from acute renal failure (30–34).

On the basis of indirect data such as sequential analysis of serum citrate variations (26, 29) and the study of the calcium fraction chelated by circulating phosphate (28), it has been suggested that this early hypercalcemia is accompanied by a decrease in serum-ionized calcium concentration, the latter stimulating parathyroid hormone secretion. The measurement of serum ionized calcium and iPTH concentrations performed in the present study support these assumptions. Furthermore, our results show for the first time to our knowledge that in this experimental model circulating concentrations of iCT are lowered after acute nephrectomy. This decrease is not due to a dilutional effect of nephrectomy, since total protein in serum is not changed. Apparently, the increased bone resorption, as evaluated by the rise in serum total calcium and specific activity of ^{45}Ca, is mediated by a stimulation of parathyroid hormone secretion and additionally caused by a fall in the serum concentration of calcitonin. The kidney is important for the clearance of both active and inactive fragments of these hormones (35, 36). Hence, it is difficult to be sure that the observed changes in circulating levels of iPTH and iCT reflect changes in the secretion of the biologically active hormones. Nevertheless, serum concentrations of iPTH were decreased and iCT normalized when serum-ionized calcium was raised by the administration of 1,25-$(OH)_2D_3$. These observations suggest that the results obtained with the immunoassays used have some biological relevance even in nephrectomized animals. Our experiments, furthermore, demonstrate an increase in serum total calcium as well as a rise in the release of previously incorporated ^{45}Ca and an increase in the number of osteoclasts even in parathyroidectomized rats in response to nephrectomy. These observations indicate that in acutely uremic rats factors other than parathyroid hormone contribute to the increase in bone resorption. The present investigation does not identify these factors. It is notable, however, that acidosis, at least at this stage of postnephrectomy, is not involved since in both parathyroid-intact and parathyroidectomized rats bilateral nephrectomy does not cause changes in blood pH.

A comparison of the effects of the two dihydroxylated metabolites of vitamin D, 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$, on the hormonal, biochemical, and bone abnormalities present at an early stage after nephrectomy demonstrates that at equimolar doses the two derivatives are highly active but exhibit different activities. Pretreatment with 1,25-$(OH)_2D_3$ increases bone resorption after bilateral nephrectomy and exaggerates the rise in serum levels of total calcium and citrate and that of the specific activity of ^{45}Ca. 1,25-$(OH)_2D_3$, furthermore, augments the serum concentration of ionized calcium and, as a consequence, lowers parathyroid hormone and increases calcitonin levels. The resorptive effect of 1,25-$(OH)_2D_3$ is observed in both parathyroid-intact and parathyroidectomized rats. These observations are in agreement with the well-established stimulating effects of this potent metabolite of vitamin D on bone resorption (37–39).

In contrast, pretreatment with 24,25-$(OH)_2D_3$ reduces the rise in serum total calcium concentration and specific activity of ^{45}Ca. It normalizes serum iPTH levels in spite of the persistance of decreased ionized calcium concentrations. A direct suppressive effect of 24,25-$(OH)_2D_3$ independent of changes in extracellular calcium concentration (11–13) is thus confirmed in the present investigation. Similarly, the rise in serum iCT levels after treatment with 24,25-$(OH)_2D_3$ does not

appear to involve modifications in serum-ionized calcium concentrations.

The inhibitory effect of 24,25-$(OH)_2D_3$ on bone calcium mobilization was seen not only in parathyroid-intact but also in rats deprived of their parathyroid glands. Hence, it can be assumed that 24,25-$(OH)_2D_3$ can act on bone in the absence of the parathyroids. This hypothesis is strenghtened by previous results obtained in rat calvaria in vitro, showing that the presence of small concentrations of 24,25-$(OH)_2D_3$ (1 nm to 1 pM) in the culture medium significantly diminishes both the stimulation of the activity of bone enzymes, such as acid phosphatases and β-glucuronidase, and the release of ^{45}Ca induced by different substances causing bone resorption i.e., parathyroid hormone, vitamin A, and heparin (16).

Furthermore, our present results demonstrate that the simultaneous administration of 24,25-$(OH)_2D_3$ partly attenuates the stimulating effect of 1,25-$(OH)_2D_3$ on bone resorption. This effect of 24,25-$(OH)_2D_3$ is observed even though equimolar doses of the two metabolites are used. Yet, since the circulating concentrations of 1,25-$(OH)_2D_3$ are ~0.1 of those of 24,25-$(OH)_2D_3$, the amount of 1,25-$(OH)_2D_3$ given to our animals is clearly much higher than the 24,25-$(OH)_2D_3$ they received. This difference, however, is compensated by the fact that the half-life of 1,25-$(OH)_2D_3$ is shorter when compared with that of 24,25-$(OH)_2D_3$, and short compared with the 16-h period between the administration of this metabolite and nephrectomy (1).

It should be noted that pretreatment with 24,25-$(OH)_2D_3$ prevents the increase in the number of osteoclasts in tibial metaphyses in parathyroid-intact rats. Even though a comparable inhibition of the release of previously incorporated ^{45}Ca is also seen in parathyroidectomized rats pretreated with 24,25-$(OH)_2D_3$, a reduction of the number of osteoclasts was not observed. This observation suggests either that the inhibitory effect of 24,25-$(OH)_2D_3$ on bone resorption involves cells other than osteoclasts, i.e., osteocytes, and/or that this metabolite suppresses the activity of the osteoclasts and not their numbers.

In conclusion, our study demonstrates the high potency of 24,25-$(OH)_2D_3$ in inhibiting the bone resorption and calcium mobilization provoked by nephrectomy. The presence of this metabolite also partly inhibits the bone resorbing action of 1,25-$(OH)_2D_3$. Whether this action is specific for 24,25-$(OH)_2D_3$ or shared by additional vitamin D metabolites cannot be established by the present study. Clearly, the effects of 24,25-$(OH)_2D_3$ are different from those of 1,25-$(OH)_2D_3$. They also differ from the effects of large doses of 25-hydroxycholecalciferol, as we have previously demonstrated that the administration of this latter metabolite to anephric rats increases the mobilization of calcium from bone (40). Whether 25,26-dihydroxycholecalciferol is able to suppress increased bone resorption as does 24,25-$(OH)_2D_3$ remains to be determined. The doses of 24,25-$(OH)_2D_3$ used in these experiments appear pharmacological relative to known existing concentrations in the normal homeostatic environment. Therefore, these observations would suggest that for effective suppression of bone resorption, pharmacological doses of this metabolite must be administered. The potential clinical significance of these data remains to be determined.

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