Local Action of Phosphate Depletion and Insulin-like Growth Factor 1 on In Vitro Production of 1,25-dihydroxyvitamin D by Cultured Mammalian Kidney Cells

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Abstract

The hormonal form of vitamin D, 1,25(OH)$_2$D, is synthesized mostly in proximal renal tubular cells. Experimental and clinical studies suggest that the growth hormone may be involved in growth-related fluctuations of plasma 1,25(OH)$_2$D and in the increase of 1,25(OH)$_2$D induced by in vivo phosphate deprivation, an action possibly mediated by insulin-like growth factor 1 (IGF 1). We tested the effects of phosphate depletion and IGF 1 addition on 1,25(OH)$_2$D$_3$ production in cultured kidney cells: opossum kidney (OK) cells, LLC-PK 1, and rabbit's proximal tubular cells.

Confluent cell monolayers were preincubated in various phosphate concentrations, in the presence and absence of IGF 1. Then, 5 nM of [^{3}H]25(OH)D$_3$ or 2 μM of 25(OH)D$_3$ were added to the medium and the cells were incubated for a further 120 min. The amount of biosynthesized 1,25(OH)$_2$D$_3$ in lipid extracts was determined after two different straight phase high performance liquid chromatographies. The experiment showed the following: (*a*) LLC-PK 1 and rabbit's cells expressed a detectable ability to synthesize 1,25(OH)$_2$D$_3$, while OK cells did not. (*b*) Partial or total phosphate deprivation increased the amount of 1,25(OH)$_2$D$_3$ produced, respectively in LLC-PK 1 and in rabbit's cells. (*c*) IGF 1 (25 ng/ml) increased 1,25(OH)$_2$D$_3$ production in rabbit's cells, particularly in phosphate-free medium (1.6-fold), and in LLC-PK 1 cells, in partial phosphate depletion (2.75-fold in 1 mM phosphate, $P = 0.015$, $n = 5$, and 3.2-fold in 0.5 mM phosphate, $P = 0.043$, $n = 4$). Our findings demonstrate a local action of phosphate depletion and of IGF 1 on 1,25-dihydroxyvitamin D$_3$ production (*J. Clin. Invest.* 1994. 94:1673–1679.) Key words: calcitriol • OK cells • LLC-PK 1 cells • rabbit's proximal tubular cells • somatomedin

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Introduction

The hormonal form of vitamin D, 1,25-dihydroxyvitamin D (1,25-[OH]$_2$D) is known to play an important role in bone mineralization during growth (1) and its circulating concentrations are the subject of fluctuations which are parallel to those of growth velocity (2–5). Physiologically, in nonpregnant mammalians, most of 1,25-(OH)$_2$D synthesis takes place in proximal tubular cells (6–10). 25-(OH)D$_3$-1αhydroxylase (1αOHase), responsible for this synthesis in the kidney, is known to be under the control of a series of modulators. Parathyroid hormone (PTH), 1,25-(OH)$_2$D$_3$, calcitonin, and extracellular concentrations of calcium and phosphate were first evidenced as modulators of this enzyme system (6–11). Phosphate is a particularly interesting regulator of 1αOHase: in animals and in humans (6, 7, 9, 10, 12, 13), dietary phosphate restriction or low phosphate serum concentrations lead to an increase of plasma 1,25-(OH)$_2$D concentration, but in vitro no direct effect of phosphate depletion on 1,25-(OH)$_2$D$_3$ production has so far been evidenced (14, 15). Moreover, in vivo, the adaptation of 1,25-(OH)$_2$D$_3$ production to phosphate depletion needs integrity of the hypothalamo–hypophysis axis (16–19).

None of the above mentioned regulating factors explains the evolution of 1,25-(OH)$_2$D synthesis during growth. The plasma concentration of 1,25-(OH)$_2$D is higher in children than in adults. But PTH is not elevated, calcium concentrations are not decreased, and phosphate concentrations are increased, and not decreased during childhood. Experimental and clinical studies suggest that the growth hormone (GH) may be involved in these growth-related fluctuations of plasma 1,25-(OH)$_2$D, and in the plasma 1,25-(OH)$_2$D increase induced by in vivo phosphate deprivation (16–19). This GH action may be mediated by the insulin-like growth factor 1 (IGF 1) (20, 21). However, the only influence of IGF 1 on 1αOHase activity evidenced in vitro was obtained at a pharmacological dose (22).

To have a better comprehension of the regulation of 1,25-(OH)$_2$D during growth, we tested the in vitro effects of phosphate deprivation and IGF 1 addition on 1αOHase activity in three different types of mammalian kidney cells in culture: opossum kidney (OK)[1] cells, LLC-PK 1, and rabbit's proximal tubular cells.

Methods

Materials. Ethanolamine, insulin, transferrin, dexamethasone, epidermal growth factor (EGF), retinoic acid, type I collagenase, sodium selenite

1. *Abbreviation used in this paper:* OK, opossum kidney.

and bovine serum albumin were purchased from Sigma Chemical Co. (St Louis, MO). Percoll was purchased from Pharmacia AB (Uppsala, Sweden). Culture media were from Eurobio (Paris, France), and reagents were from Flow Laboratories (Irvine, U.K.). Plasticware was from Falcon (Oxnard, CA).

1,25-$(OH)_2D_3$ was a gift from Roche Laboratories (Basel, Switzerland), [26,27-^{3}H]25-$(OH)D_3$ (specific activity 19 Ci/mmol) and [26,27-^{3}H]1,25-$(OH)_2D_3$ (specific activity 110 Ci/mmol) were purchased from Amersham (Amersham, U.K.) and unlabeled 25-$(OH)D_3$ was a gift from Roussel Laboratories (Paris, France). Chromatography solvents (n-hexane, isopropranol, methylene chloride) were of HPLC-grade and purchased from Merck (Germany). Scintillation fluid was purchased from Packard Instruments (Downers Grove, IL).

Insulin-like Growth Factor 1 (IGF 1) was human recombinant and purchased from Becton Dickinson Labware (France).

Kidney cell cultures. The cultured kidney monolayers used in this study were of three types: (*a*) A renal cell line derived from an opossum kidney (OK) as originally established by Koyama et al. (23). The cells, between the 60th and 70th passages, were cultured in a DME-Ham F12 mixture containing L-glutamine, 2.5% FCS, 50 IU/ml penicillin, and 50 μg/ml streptomycin as previously described (24). All cultures were maintained at 37°C under 5% CO_2 in air. The cells were plated in 24-well plastic trays. They reached confluence within 4 d. The assays were performed on day 5–6.

(*b*) LLC-PK 1 cells (passages 200–220, obtained from Flow Laboratories) were seeded in 24-well plastic trays and grown at 37°C under 5% CO_2 in a serum-free medium. The medium consisted of a 1:1 (vol/vol) Ham's F-12 and DMEM supplemented as previously described (25).

(*c*) Primary cultures of rabbit's renal proximal tubular cells were prepared according to Bello-Reuss and Webber (26) with minor modifications (27). Kidneys were removed aseptically from anesthetized New Zealand rabbits, decapsulated and sliced in 1-mm-thick sections. Cortex was separated from medulla, cortical slices were rinsed and submitted to trypsine-collagenase digestion for 50–60 min. Then, the mixture of renal tubules was washed and centrifugated. Homogeneous populations of nephron segments were separated by Percoll centrifugation as described by Vinay et al. (28). The F4 layer, made of proximal tubules, was removed, suspended in HBS-Hepes, washed and centrifugated three times in this solution. The final pellet was suspended in the culture medium and tubules were seeded in 24-well plastic trays coated with NH_3-reconstituted rat's tail collagen (2.5×10^4 fragments/well) (29). The serum-free culture medium (30) consisted in a 1:1 (vol/vol) Ham's F-12 and DME mixture supplemented with 5 μg/ml insulin, 35 μg/ml transferrin, 10 ng/ml epithelial growth factor (EGF), 2.2 μM retinoic acid, 20 μM ethanolamine, and 50 nM dexamethasone. Cells were first seeded with a medium containing 1% of fetal calf serum (FCS), and cultured after the third day in a serum-free medium.

Metabolism of 25-(OH)D3 by cells in vitro. On the day before the experiment, the culture medium was changed and the cells (~ 50,000 cells/well) were preincubated for 16 h in 0.5 ml of a hormone and phosphate free DMEM with or without 0.5, 1, or 2 mM KH_2PO_4 and with or without 25 ng/ml IGF 1. There was no increase of cell protein concentration after the 16-h incubation with IGF 1.

To measure the ability of these cells to convert 25-$(OH)D_3$ into 1,25-$(OH)_2D_3$, 10 μl of ethanol containing 50 nCi [^{3}H]25-$(OH)D_3$ (final concentration, 5 nM) or 10 μl of ethanol containing 0.4 μg 25-$(OH)D_3$ (final concentration, 2 μM) were added to the medium and the incubation was continued for 120 min. Cells and medium were then transferred to glass tubes and the reaction was terminated by a Bligh and Dyer (31) extraction: 1 ml of HPLC grade methanol and 1 ml of chloroform were added to each sample. The chloroform phase was dried under N_2, and the residue redissolved in the chromatographic solvent. The samples were cochromatographed with either 100 ng unlabeled 1,25-$(OH)_2D_3$, for radioactive cell extracts, or 3,000 dpm [^{3}H]1,25-$(OH)_2D_3$, for unlabeled cell extracts, using a straight phase HPLC system and a 4.6 × 250-mm Ultrasphere Si column (Altex, Berkeley, CA), equilibrated with *n*-hexane:isopropanol (92:8, flow rate 1.5 ml/min). Absorbance at 254 nm was continuously monitored and fractions of the effluent were collected every 1 min for determination of radioactivity. Fractions co-eluting with synthetic 1,25-$(OH)_2D_3$ (or [^{3}H]1,25-$[OH]_2D_3$) were pooled and rechromatographed as described above but using a methylene chloride:isopropanol (95:5, flow rate 1 ml/min) solvent system. For incubations performed in the presence of 5 nM [^{3}H]25-$(OH)D_3$, the rate of conversion of [^{3}H]25-$(OH)D_3$ into [^{3}H]1,25-$(OH)_2D_3$ was determined by calculating the percentage of total radioactivity with an appropriate elution profile after the two successive chromatographies. Results are expressed as fmol/10^6 cells, based on the assumption that the specific activity of the product was the same as that of the substrate. Thus, conversion of 1% of the substrate into [^{3}H]1,25-$(OH)_2D_3$ corresponds to the production of 25 fmol.

The purified 25-$(OH)D_3$ metabolite produced during incubations of kidney cells with 2 μM unlabeled 25-$(OH)D_3$ was tested for its ability to bind to the 1,25-$(OH)_2D_3$ receptors present in chick intestinal cytosol using the radiocompetition binding assay used to measure plasma 1,25-$(OH)_2D$ (32).

The amount of 1,25-$(OH)_2D_3$ produced during the incubation with 0.4 μg 25-$(OH)D_3$ was calculated from the absorbance at 254 nm of the single peak co-eluting with synthetic [^{3}H]1,25-$(OH)_2D_3$ in the second HPLC sytem.

Ultraviolet (UV) absorption spectra of the purified metabolite were performed using a HPLC system fitted with a photodiode array detector (model 996; Millipore/Continental Water Systems, Saint Quentin en Yvelines, France), and compared with the spectrum of synthetic 1,25-$(OH)_2D_3$ (Millenium 2010).

Statistical methods. Statistical analyses were performed using the t-test or the Wilcoxon test for paired data, with and without phosphate depletion, with and without IGF 1, (Statworks; Microsoft corporation, Seattle, WA).

Results

Metabolism of 25-(OH)D_3 by renal cells in vitro. The three types of mammalian kidney cells studied were able to convert 25-$(OH)D_3$ into more polar metabolites. With LLC-PK 1 and rabbit's cells one of these metabolites eluting as a single peak coeluted with synthetic 1,25-$(OH)_2D_3$ on straight phase HPLC using a 92:8 n-hexane:isopropanol solvent (Fig. 1 *A*) and using a 95:5 methylene chloride:isopropanol solvent (Fig. 1 *B*). The amount of this metabolite found after 120 min of incubation in a 2 mM phosphate medium with 5 nM [^{3}H]25-$(OH)D_3$ ranged between 20 and 1100 fmol/10^6 cells in rabbit's cell cultures (mean = 363 fmol/10^6 cells, n = 10), and between 40 and 500 fmol/10^6 cells (mean = 151, n = 8) in LLC-PK 1 (Table I). The amount produced during 120 min incubation with 2 μM 25-$(OH)D_3$ ranged between 50 and 150 pmol/10^6 cells and was sufficient to show that the 1,25-$(OH)_2D_3$-like metabolite absorbed UV light, with a UV spectrum identical to that of synthetic 1,25-$(OH)_2D_3$ (maximal absorbance at 265.8 nm, match angle = 3.851), and that its ability to compete with the [^{3}H]1,25-$(OH)_2D_3$ for binding to the chick intestinal cytosol was identical to that of synthetic 1,25-$(OH)D_3$ (Fig. 2). In contrast, with OK cells, the metabolite coeluting with synthetic 1,25-$(OH)_2D_3$ in the first HPLC system (Fig. 1 *C*) did not coelute with the synthetic hormone in the second HPLC system (Fig. 1 *D*).

Effect of phosphate deprivation on 1,25-$(OH)_2D_3$ synthesis. In vitro phosphate deprivation of cultured rabbit's kidney cells for 16 h increased their ability to produce 1,25$(OH)_2D_3$ (Fig. 3). The amount of 1,25$(OH)_2D_3$ produced after 120 min incubation was 3.8±1.1-fold higher (mean±SE, n = 6) in rabbit's

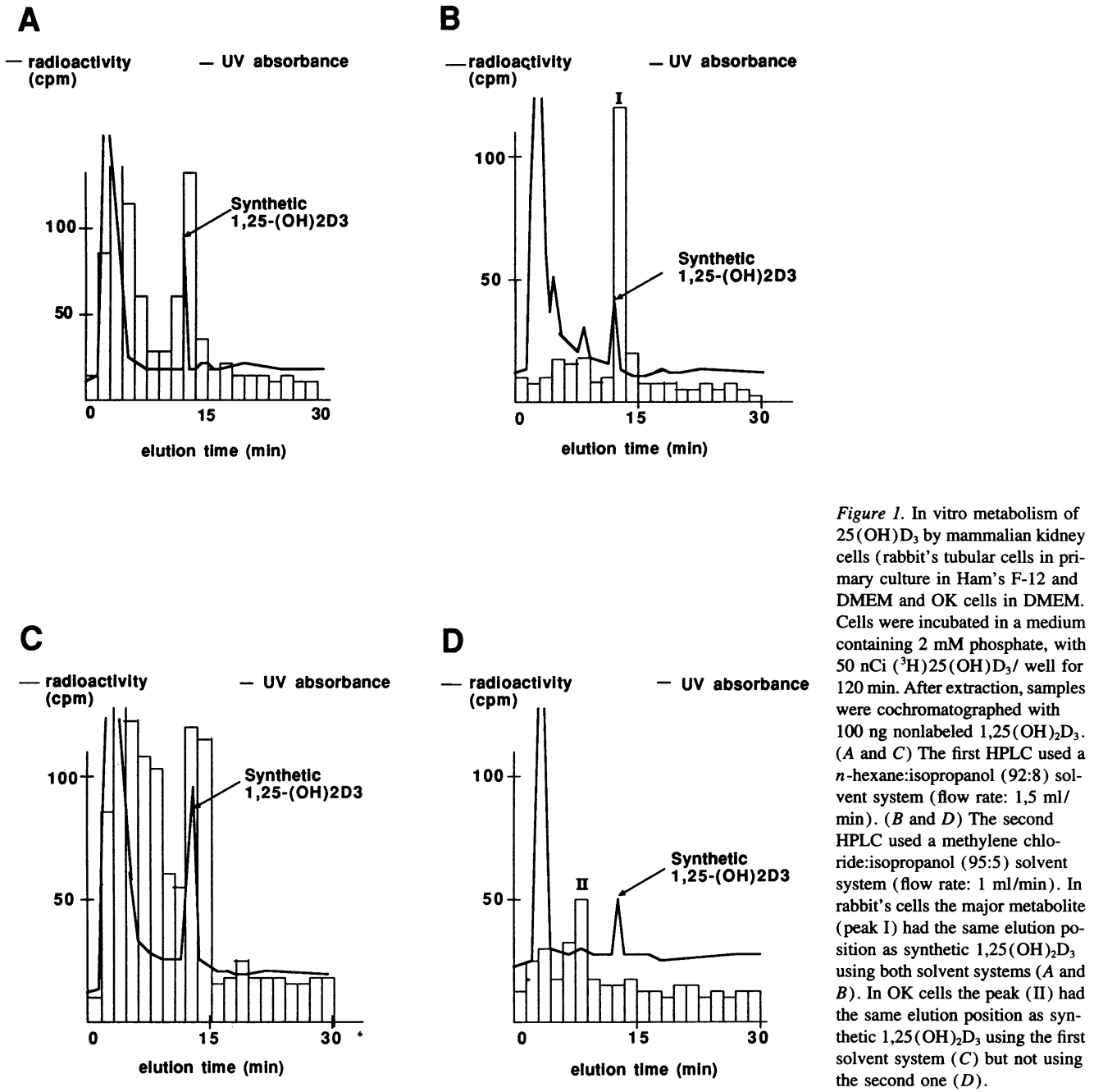

Figure 1. In vitro metabolism of 25(OH)D_3 by mammalian kidney cells (rabbit's tubular cells in primary culture in Ham's F-12 and DMEM and OK cells in DMEM. Cells were incubated in a medium containing 2 mM phosphate, with 50 nCi (^{3}H)25(OH)D_3/ well for 120 min. After extraction, samples were cochromatographed with 100 ng nonlabeled 1,25$(OH)_2D_3$. (*A* and *C*) The first HPLC used a *n*-hexane:isopropanol (92:8) solvent system (flow rate: 1,5 ml/min). (*B* and *D*) The second HPLC used a methylene chloride:isopropanol (95:5) solvent system (flow rate: 1 ml/min). In rabbit's cells the major metabolite (peak I) had the same elution position as synthetic 1,25$(OH)_2D_3$ using both solvent systems (*A* and *B*). In OK cells the peak (II) had the same elution position as synthetic 1,25$(OH)_2D_3$ using the first solvent system (*C*) but not using the second one (*D*).

cells incubated in a phosphate free medium (mean = 915, range: 107–4220 fmol/10^6 cells) than in the presence of 2 mM phosphate (mean = 272, range: 20–1100 fmol/10^6 cells), P = 0.014.

The stimulatory effect of phosphate depletion appeared to be related to a higher rate of 1,25$(OH)_2D_3$ production rather than to a decreased rate of 1,25$(OH)_2D_3$ catabolism, for it was observed as early as 30 min after the addition of 25(OH)D_3 to the cell medium.

In LLC-PK 1 cells, partial phosphate depletion (1 mM) for 16 h led to a significant (2.26±fold) stimulation of 1,25$(OH)_2D_3$ production, from 128 (range: 40–500) fmol/10^6 cells/120 min (n = 7) to 231 (range: 62–650) fmol/10^6 cells/120 min (n = 7). In contrast, 1,25$(OH)_2D_3$ synthesis was not stimulated in cells submitted to more severe phosphate depletion (0.5 mM) and 1,25$(OH)_2D_3$ was undetectable in cells submitted to total phosphate depletion (Fig. 4).

Effect of IGF 1 adjunction and adaptation to different extracellular concentrations of phosphate. Incubation of rabbit's cells with 25 ng/ml human IGF 1, for 16 h, led to a limited and inconstant (3 out 5 experiments) increase in 1,25$(OH)_2D_3$ production when cells had been cultured in a serum and hormone free medium containing 2 mM phosphate (Fig. 3). Yet, it had a marked stimulatory effect in cells incubated in a phosphate free medium. This effect was observed as early as 30 min after the addition of 25(OH)D_3 to the medium (Fig. 3) and led

Table I. [^3H]1,25(OH)$_2$ Vitamin D_3 Synthesized by Cultured Kidney Cells after a 120-min Incubation in a 2-mM Phosphate Medium in Presence of 5 nM [^3H]25(OH)D_3

Cellular type	[^{3}H]1,25(OH)$_2$D$_3$ biosynthesized (fmol/10^6 cells/120 min) Mean (range)	Experiments
OK	0	$n = 5$
LLC-PK1	151 (40–500)	$n = 8$
Rabbit's tubular cells	363 (20–1100)	$n = 10$

to a 1.6-fold increase after 120 min, from 915 (range: 107–4220) fmol/10^6 cells/120 min to 1670 (range: 101–7230) fmol/10^6 cells/120 min ($n = 5$). Similarly, incubation of LLC-PK 1 cells with 25 ng/ml human IGF 1, for 16 h, led to a limited, non significant increase in 1,25(OH)$_2$D$_3$ production

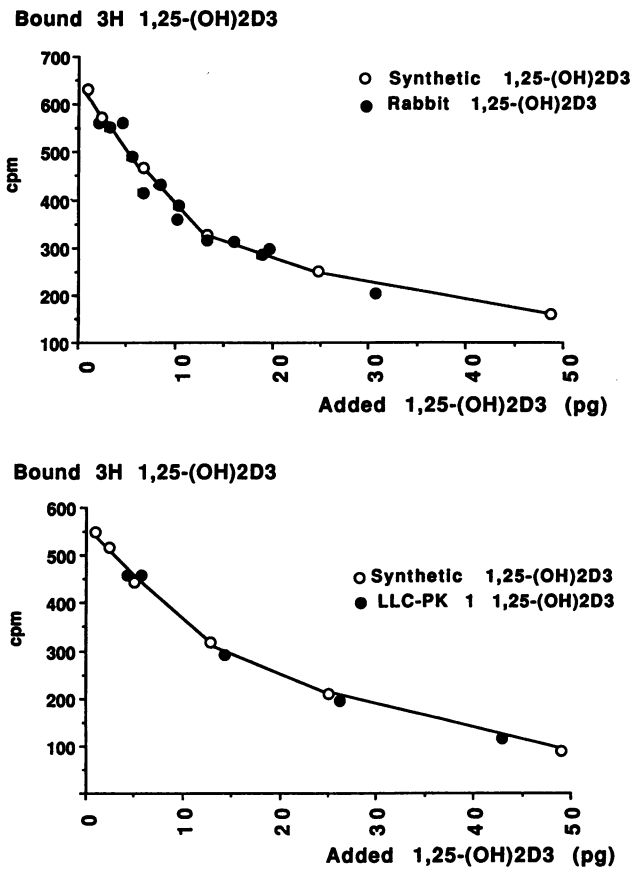

Figure 2. Ability of metabolites of 25(OH)D$_3$ produced by mammalian kidney cells in culture to bind to 1,25(OH)$_2$D$_3$ receptors. Metabolites of nonlabeled 25(OH)D$_3$ produced in vitro were purified by two successive HPLC, and the ability of various concentrations of these metabolites to compete with the binding of synthetic (^{3}H)1,25(OH)$_2$D$_3$ to receptors present in chick intestinal cytosol was tested. Shown are synthetic 1,25(OH)$_2$D$_3$ (○) and metabolites produced by cultured cells (●). Rabbit's tubular cells on the top, and LLC-PK 1 on the bottom. Each closed circle represents the mean of two experiments performed in triplicate. Standard errors were < 5%.

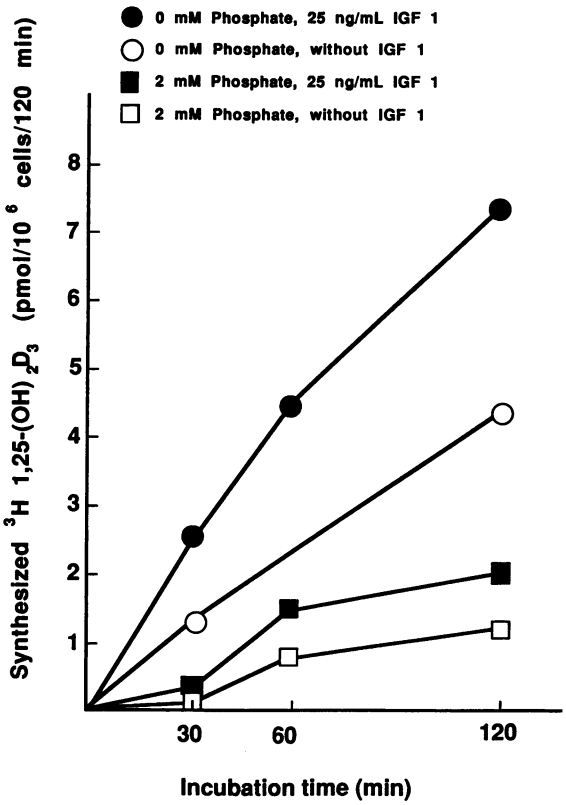

Figure 3. Effects of IGF 1 and medium phosphate concentration on 1,25(OH)$_2$D$_3$ synthesis in rabbit's tubular cells in primary culture. Time course: cells were incubated with 50 nCi [^{3}H]25(OH)D$_3$/well for 0, 30, 60, and 120 min. The experiments have been done after a 16-h preincubation in a 0 mM phosphate medium (*circles*) or in a 2 mM phosphate medium (*squares*), without (*open*) or with (*closed*) 25 ng/ml IGF 1. Metabolites have been purified using two successive HPLC and the one with the same elution position as synthetic unlabeled 1,25(OH)$_2$D$_3$ in both systems has been quantified.

when cells had been cultured in a serum and hormone free medium containing 2 mM phosphate, but to a marked increase in a partially phosphate depleted medium: 2.75-fold in 1 mM phosphate ($P = 0.015$, $n = 5$) and 3.2-fold in 0.5 mM phosphate ($P = 0.043$, $n = 4$), (Fig. 4).

Discussion

We have shown that two types of mammalian kidney cells in culture (LLC-PK 1 and rabbit's tubular cells) are able to synthesize 1,25-(OH)$_2$D$_3$, and that this synthesis is modulated by local conditions, particularly extra-cellular phosphate concentrations and presence of IGF 1 in the medium.

Up to now several types of kidney cells have been studied in vitro for their ability to produce 1,25-(OH)$_2$D$_3$: kidney cell homogenates, chick kidney cells in primary culture (33–38), and mammalian kidney cells (15, 39). With these cells, a direct effect of PTH, 1,25-(OH)$_2$D$_3$, and calcitonin has been evidenced on 1,25-(OH)$_2$D$_3$ production, but no direct effect of physiological doses of IGF 1, or phosphate depletion had yet been demonstrated. The rationale for using the three other cell types studied in the present work is the following: (*a*) OK cells,

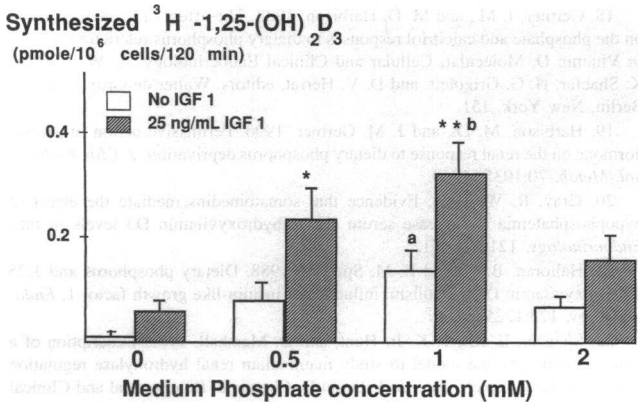

Figure 4. Effects of IGF 1 and phosphate concentration in culture medium on 1,25(OH)$_2$D$_3$ synthesis in LLC-PK 1 cells. The cells were preincubated for 16 h in various phosphate concentrations (0, 0.5, 1, 2 mM) without (□) or with (■) 25 ng/ml IGF 1. The cells were incubated with 50 nCi [^{3}H]25(OH)D$_3$/well for 120 min. Metabolites of [^{3}H]-25(OH)D$_3$ have been purified using two successive HPLC and the one with the same elution position as synthetic 1,25(OH)$_2$D$_3$ in both systems has been quantified. Each bar represents the mean±SE of four or five experiments. (*) Effect of IGF 1 in 0.5 mM phosphate medium, $P = 0.043$, $n = 4$; (**) effect of IGF 1 in 1 mM phosphate medium, $P = 0.015$, $n = 5$; (*a*) in comparison with 2 mM phosphate, without IGF 1, $P = 0.038$, $n = 5$; (*b*) in comparison with 2 mM phosphate, with IGF 1, $P = 0.013$, $n = 5$.

obtained from an opossum kidney, were considered as tubular proximal cells because of their Pi uptake and are especially responsive to PTH, which decreases their Pi uptake and stimulates AMPc synthesis (40); (*b*) LLC-PK 1 cells, derived from a pig kidney, are known to express 1,25-(OH)$_2$D$_3$ receptors (41), and a 25-(OH)D$_3$-24-OHase responding to 1,25-(OH)$_2$D$_3$, they also have a Na dependent-Pi uptake, but are non sensitive to PTH (40); (*c*) Rabbit's cells in primary culture are tubular proximal cells (42, 43): they are epithelial, oriented with a brush border, they have Na dependent uptake of phosphate, glucose, and aminoacids and they express an AMPc response to PTH but not to calcitonin.

In our study, OK cells did not express an ability to synthesize 1,25(OH)$_2$D$_3$. This lack of expression may result from the conditions used in this study, or may be an argument in favor of the pars recta origin of the OK cells, as the 25-(OH)D$_3$-1αOHase has been mainly localized in the proximal convoluted tubules in chick and rat kidneys (8). In contrast, LLC-PK 1 cells and rabbit's cells in primary culture expressed a clear 25-(OH)D$_3$-1αOHase activity, and thus proved to be new useful models for studying the 1αOHase enzymatic system. Indeed, these cells proved to be able to convert 25-(OH)D$_3$ into a metabolite which shared with 1,25-(OH)$_2$D$_3$ identical chromatographic properties on two HPLC systems, similar UV absorbance at 254 nm, identical UV spectrum, and identical ability to compete with [^{3}H]1,25-(OH)$_2$D$_3$ for binding to the chick intestinal cytosol. The only vitamin D metabolite known to meet these criteria is 1,25-(OH)$_2$D$_3$ (32, 44). These results also show that the ability to synthesize 1,25-(OH)$_2$D$_3$ and the sensitivity to PTH (cAMP dependent) do not necessarily coexist in the same cultured renal cell line. LLC-PK 1 cells were able to produce 1,25(OH)$_2$D$_3$ in our experimental conditions but are known not to be responsive to PTH, while OK cells were unable to synthesize 1,25(OH)$_2$D$_3$ but are responsive to PTH (40).

Using these mammalian cell models, we were able to show a local effect of phosphate and IGF 1. Phosphate plays a physiological role in the regulation of serum concentration of 1,25(OH)$_2$D$_3$: oral intake and serum concentration of phosphorus alter serum concentration of 1,25(OH)$_2$D$_3$ and its production rate (12, 13), via a possibly GH- or IGF 1–dependent mechanism (16–21, 45). The results of our study provide the first evidence of a local action of phosphate concentration on 1,25(OH)$_2$D$_3$ production, not requiring exogenous IGF I: total phosphate depletion in rabbit's kidney cells and partial phosphate depletion in LLC-PK 1 had a clear stimulating effect on 1,25(OH)$_2$D$_3$ production. Trechsel et al. (14) in 1979 did not evidence this effect of phosphate depletion while studying cultured chick kidney cells. Two main differences in the experimental protocols may explain these discrepancies: differences in the species origin of the cells, or the use by Trechsel et al. (14) of a medium enriched with 10% FCS which may have masked the phosphate effect, while we used a serum and hormone-free medium. Fukase et al. (15) in 1982 found an inhibitory effect of high phosphate concentrations on the ability of the mouse kidney cells to produce 1,25(OH)$_2$D$_3$, but did not detect any effect of phosphate depletion (15). They concluded that the effect of phosphate deprivation is only indirect. But they used an insulin (5 μg/ml) enriched medium to study 1αOHase and an interaction between this hormone and phosphate can not be excluded.

While partial phosphate depletion (1 mM) of LLC-PK 1 cells stimulated their ability to produce 1,25(OH)$_2$D$_3$, total phosphate depletion led to an almost complete loss of this ability. Complete phosphate depletion can deeply affect cell metabolism and the phosphorylation–dephosphorylation pathway regulating 25-(OH)D$_3$-1αOHase activity. Previous studies reported that LLC-PK 1 cells cultured in a phosphate free medium exhibit an increase of Na dependent phosphate uptake, an unaltered ATPase but a sharp decrease of intracellular phosphate and ATP (46).

IGF 1 is thought to be the messenger of phosphate depletion. In vivo studies have shown its action on phosphate mediated control of 1,25(OH)$_2$D$_3$ production (20, 21, 45). But up to now, the only reported in vitro stimulating effect of IGF 1 on 1,25(OH)$_2$D$_3$ production was obtained with a pharmacological concentration of Somatomedin C by Spencer et al. (22). Our results clearly show that low concentrations of exogenous IGF 1 can stimulate 1,25(OH)$_2$D$_3$ synthesis. This effect has not been evidenced before, probably because of the media that were used: they contained serum (and therefore IGF and IGF BPs), or insulin (which can saturate IGF 1 receptors). In keeping with what has been observed after its in vivo administration (47), IGF 1 added directly in vitro had only a limited effect on 1,25(OH)$_2$D$_3$ synthesis when phosphate was at a normal level, but had a marked effect when phosphate concentration in the medium was low. Thus, IGF 1 appears to be an important regulator of the adaptation of 1,25(OH)$_2$D$_3$ synthesis to phosphate deprivation, although such adaptation does not require the presence of exogenous IGF 1.

Phosphate concentration and IGF 1 may act at different levels on the 1αOHase system. Phosphate depletion may directly enhance dephosphorylation of the ferredoxin, thus activating the 1αOHase (48, 49). IGF 1 may potentiate the phos-

phate depletion effect through direct action on phosphate captation (50–52). It may also stimulate the synthesis of the cytochrome P450 component of the enzyme complex. It may finally affect dephosphorylation of the ferredoxin through a PKC mediated pathway (53).

Whatever the mechanism involved, this first demonstration of a local effect of IGF 1 on $1,25(OH)_2D_3$ synthesis suggests that IGF 1 can have a double function during growth: not only of accelerating growth, but also of increasing bone mineralization through regulation of $1,25(OH)_2D_3$ production.

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