REGULATION OF 25-OHD$_3$ METABOLISM BY PARATHYROID HORMONE IN PRIMARY CHICK KIDNEY CELL CULTURES

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1. Introduction

The regulation of 1,25-dihydroxycholecalciferol (1,25(OH)$_2$D$_3$) production has been the most hotly debated question in vitamin D metabolism. Despite the controversies of the past few years it is now agreed that the regulation of 1,25(OH)$_2$D$_3$ production is multifactorial. The available evidence indicates that the production of this hormone is influenced by ionic and hormonal factors such as calcium [1], phosphate [2], parathyroid hormone [3], prolactin [4], sex steroids [5], growth hormone [6] and 1,25(OH)$_2$D$_3$ [7] itself.

Until recently, experimental approaches to elucidate the regulation of vitamin D metabolism have relied almost exclusively upon the use of whole animal systems. The inherent inability of in vivo approaches to differentiate between primary and secondary effects makes interpretation of the results not only difficult but also inconclusive and therefore controversial. Secondary changes can be eliminated by studies in vitro. We have shown that primary chick kidney cell culture is a very sensitive in vitro system to study the regulation of vitamin D metabolism [8]. Using this system we have been able to show that parathyroid hormone acts directly to regulate *both* renal 25-OHD$_3$ hydroxylases.

2. Materials and methods

Culture medium (Eagle's minimum essential medium with Earle's salts), foetal calf serum, trypsin, tryptose phosphate broth and antibiotic solution (penicillin and streptomycin) were purchased from Gibco-Biocult, Paisley, Renfrewshire, Scotland. Bovine parathyroid hormone (2500 U/mg) was a gift from the National Institute for Biological Standards, Hampstead. Radiolabelled 25-hydroxy-[26,27-*Me*-^{3}H]-cholecalciferol, spec. act. 9 Ci/mmol, was supplied by the Radiochemical Centre, Amersham, Bucks. Crystalline synthetic 1,25(OH)$_2$D$_3$ was a gift from Dr N. T. Pollitt, Roche Products, Welwyn Garden City, Herts.

2.1. *Cell cultures*

Cockerels (3–4 weeks; Rhode Island Red X Light Sussex crossbread; Orchard Farm, Great Missenden, Bucks) fed on a vitamin D-deficient diet (1.3% calcium, 1.1% phosphorus) from the day of hatching, were used to prepare primary kidney cell cultures as in [8]. When confluent, the cells were maintained in a serum-free medium for at least 24 h before they were used for experimental purposes. Some cultures were made vitamin D-replete by treatment for 20–22 h with 10 ng 1,25(OH)$_2$D$_3$/ml and were subsequently maintained in media containing 100 pg 1,25(OH)$_2$D$_3$/ml. For acute experiments, cells were treated for 1 h with parathyroid hormone or the solvent alone, while for chronic experiments the cells were exposed every 6 h for 24 h to fresh prewarmed medium containing either parathyroid hormone or the solvent alone. Parathyroid hormone was dissolved in 0.001 M acetic acid. Enzyme assays, extraction of lipids, chromatography on Sephadex LH-20 columns and identification of the vitamin D metabolites were carried out as in [8].

3. Results

The ability of this in vitro system to respond to $1,25(OH)_2D_3$ enabled us to manipulate the activity of the renal hydroxylases in such a way as to produce enzyme patterns similar to the in vivo situations. Vitamin D-deficient cells had only 1α-hydroxylase activity (table 1) and those treated with $1,25(OH)_2D_3$ possessed both 1α- and 24-hydroxylase activities (table 2). Parathyroid hormone had neither acute nor long-term effects on 1α-hydroxylase activity in vitamin D-deficient cells (table 1). No acute effects of parathyroid hormone were observed on either 1α- or 24-hydroxylase activity in vitamin D-treated cells (table 2, expt I). In contrast chronic treatment with parathyroid hormone caused a marked inhibition in 24-hydroxylase and a modest but statistically significant stimulation in 1α-hydroxylase activity (table 2, expt II and fig.1).

Table 1
Acute (expt I) and long-term (expt II) effects of bPTH on 1α-hydroxylase activity in vitamin D-deficient primary chick kidney cell cultures

PTH (ng/ml)	No. est.	1α-OHase activity (pmol $1,25(OH)_2D_3$ .flask^{-1} .h^{-1})
Expt I		
0	5	5.3 ± 1
5	5	5.7 ± 0.5
500	5	5.8 ± 0.6
Expt II		
0	5	3.7 ± 0.5
5	4	3.6 ± 0.2
500	4	2.8 ± 0.4

In expt I cells were treated for 1 h with PTH. In expt II cells were exposed every 6 h for 24 h to fresh prewarmed medium containing the indicated PTH concentrations

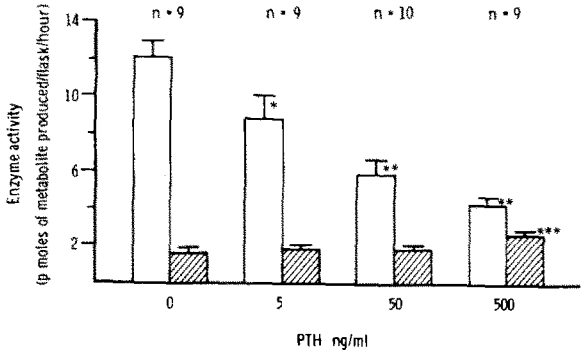

Fig.1. Long-term effects of parathyroid hormone on the renal 25-OHD_3 hydroxylases in primary chick kidney cell cultures treated with $1,25(OH)_2D_3$. Open bars represent 24-hydroxylase and cross hatched bars 1α-hydroxylase activity. n = number of estimations. Significantly different from appropriate control: * $p < 0.025$; ** $p < 0.001$; *** $p < 0.01$.

Table 2
Acute (expt I) and long-term (expt II) effects of bPTH on the renal 25-OHD_3 hydroxylases in primary chick kidney cell cultures treated with $1,25(OH)_2D_3$

PTH (ng/ml)	No. est.	Enzyme activity (pmol metabolite produced .flask^{-1} .h^{-1})	
		24-OHase	1α-OHase
Expt I			
0	4	6.6 ± 0.9	3.0 ± 0.5
5	5	6.5 ± 0.5	3.2 ± 0.2
500	5	5.8 ± 0.8	3.1 ± 0.5
Expt II			
0	5	5.2 ± 0.6	2.7 ± 0.4
5	4	3.0 ± 0.4[c]	4.1 ± 0.4[b]
500	5	1.5 ± 0.6[d]	3.8 ± 0.4[a]

Significantly different from appropriate control: [a] $p < 0.05$; [b] $p < 0.025$; [c] $p < 0.01$; [d] $p < 0.001$

4. Discussion

The realization that $1,25(OH)_2D_3$ is a very potent calcium mobilizing hormone gave impetus to investigation of the physiological mechanisms controlling its secretion by the kidney. Parathyroid hormone was one of the first factors to be implicated in the regulation of 1α-hydroxylase, the enzyme responsible for the production of $1,25(OH)_2D_3$ [3,9]. The evidence in favour of a regulatory role of parathyroid hormone in $1,25(OH)_2D_3$ production stems mainly from ablation experiments in vitamin D-replete animals. It has been shown in animals fed a low calcium vitamin D-supplemented diet, that parathyroidectomy suppresses the production of $1,25(OH)_2D_3$ and stimulates the production of $24,25(OH)_2D_3$. Administration of parathyroid hormone restores those enzyme activities to normal, over a period of several hours [3,9].

In contrast in vitamin D-deficient animals, in which only 1α-hydroxylase is present, parathyroid hormone has no effect [10,11]. Our findings in vitro are in very good agreement with these in vivo observations. No acute effects of parathyroid hormones were observed in either vitamin D-deficient or vitamin D-replete cell cultures. Moreover, chronic treatment with this hormone did not affect the 1α-hydroxylase activity in vitamin D-deficient cells, whereas in vitamin D-replete cells it produced a marked suppression of 24-hydroxylase and a modest stimulation in 1α-hydroxylase activity. This is to our knowledge the first demonstration that parathyroid hormone acts directly on the kidney cells to regulate the activities of both hydroxylases in a fashion similar to that observed in experiments in vivo. Others have also reported that chronic treatment with parathyroid hormone suppresses the 24-hydroxylase activity in primary kidney cell cultures and cell lines from mammalian species [12,13]. It is interesting to note that these cells lacked 1α-hydroxylase activity even though they were cultured for several days in the absence of any form of vitamin D.

In 1972 it was claimed that parathyroid hormone stimulates acutely the 1α-hydroxylase activity in isolated renal tubules from vitamin D-deficient chicks [14]. This observation not only is difficult to reconcile with in vivo findings, but it has not been reproduced [15,16]. The failure to show an effect of chronic treatment with parathyroid hormone on the 1α-hydroxylase activity in vitamin D-deficient chick kidney cell cultures [17] is in agreement with our observations.

Our results show that PTH has a more marked effect on 24-hydroxylase than on 1α-hydroxylase. This may be due to the experimental conditions. Because we used initially a high concentration of $1,25(OH)_2D_3$ to suppress the 1α-hydroxylase and to induce the 24-hydroxylase, it is possible that parathyroid hormone was able to suppress the 24-hydroxylase more readily than to overcome the suppressive effect of $1,25(OH)_2D_3$ on the 1α-hydroxylase.

Such an explanation seems to be supported by the in vivo observations [18]. These authors have shown that in vitamin D-deficient chicks treated with a single injection of 130 ng $1,25(OH)_2D_3$, parathyroid hormone suppressed the 24-hydroxylase but had no effect on 1α-hydroxylase activity. An alternative explanation may be that the 24-hydroxylase is more sensitive to the effects of parathyroid hormone than the 1α-hydroxylase. In either case, the fact remains that parathyroid hormone has a profound influence on 24-hydroxylase, but the physiological significance of this regulatory effect is at present unknown.

The reason for the failure of PTH to stimulate the 1α-hydroxylase activity in either vitamin D-deficient animals or cells is unclear. It may be that parathyroid hormone cannot further stimulate the 1α-hydroxylase due to the fact that the activity of this enzyme is already very high as a result of vitamin D-deficiency. By the same token, it may be argued that vitamin D-supplementation by suppressing the 1α-hydroxylase activity, enables the stimulatory effect of parathyroid hormone on this enzyme to become apparent. Others have suggested that $1,25(OH)_2D_3$ is required not only for the appearance of 24-hydroxylase, but also to induce a change in the renal cell which in turn permits regulation of the hydroxylases by parathyroid hormone [10,11,19]. But, recent work in mammalian cell cultures indicates that neither the presence of $1,25(OH)_2D_3$ nor 1α-hydroxylase are required for parathyroid hormone to regulate the 24-hydroxylase activity [12,13]. By contrast studies carried out in vivo [3,9] as well as our observations in primary chick kidney cell cultures indicate that parathyroid hormone stimulates the 1α-hydroxylase activity only under situations in which both enzymes

are present. Therefore, it is conceivable that the primary effect of parathyroid hormone may be the suppression of 24-hydroxylase leading to greater availability of substrate for 1α-hydroxylase and thus to increased production of $1,25(OH)_2D_3$.

The physiological function of $1,25(OH)_2D_3$ is largely unknown, let alone the regulation of its production. It has been suggested that in the bird and lower species the 24-hydroxylation developed as an inactivation mechanism for vitamin D and that $24,25(OH)_2D_3$ represents an excretory route for vitamin D_3 [20]. In mammals, $24,25(OH)_2D_3$ is also less active than $1,25(OH)_2D_3$ in terms of calcium mobilizing activity [21,22]. However, recent studies have led to claims that $24,25(OH)_2D_3$ plays a significant role in bone calcification [23,24]. If these claims are confirmed, then the demonstration that the production of $24,25(OH)_2D_3$ is regulated by parathyroid hormone may provide an unsuspected insight into the regulation of calcium homeostasis.

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