THE 25-HYDROXYCHOLECALCIFEROL-1-HYDROXYLASE ACTIVITY OF CHICK KIDNEY CELLS: DIRECT EFFECT OF PARATHYROID

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1. Introduction

The active vitamin D metabolite, 1,25-dihydroxycholecalciferol ($1\alpha,25(OH)_2D_3$) is the product of the hydroxylation of 25-hydroxycholecalciferol ($25(OH)D_3$) in the kidney [1–4]. Indirect evidence obtained from in vivo studies support the involvement of parathyroid hormone in regulating the activity of the enzyme which catalyzes this hydroxylation, 25-hydroxycholecalciferol-1-hydroxylase (1-hydroxylase) [1,2,5,6].

An increased production of $1\alpha,25(OH)_2D_3$ was demonstrated in kidney cells in vitro within 15 min, in response to the presence of bovine parathyroid hormone (BPTH) in the incubation medium [7]. An increased production of $1\alpha,25(OH)_2D_3$ was demonstrated in a kidney cell culture [8], in response only to much higher concentrations of BPTH in the medium and as late as after 6 h incubation. However, in kidney cell culture [9] and with clumps of intact tubular cells in vitro [10] no stimulation of 1-hydroxylase by BPTH could be demonstrated. A marked inhibition by BPTH of 24,25-dihydroxycholecalciferol ($24,25(OH)_2D_3$) production was demonstrated in kidney cells in culture, with only a modest stimulation of $1\alpha,25(OH)_2D_3$ production [11].

Here we measured the effect of some sources of parathyroid activity on the production of $1\alpha,25(OH)_2D_3$ by avian kidney cells in vitro.

2. Experimental

Kidney cell suspensions were taken from 5-week-old vitamin D-deficient or cholecalciferol-fed (14 days prior to sacrifice) chicks and prepared by a procedure similar to that in [9] but without any trypsin. The cells were resuspended and washed several times before final suspension in Eagle's (GIBCO) medium without HCO_3 (containing 20 mM Hepes and 1.0 mM $CaCl_2$) and with 0.1% bovine serum albumin (Sigma).

The viability of the kidney cells, as estimated by trypan blue exclusion, was usually ~90%. The number of cells remained unchanged during 3 h incubation; however, the viability decreased slightly to 85%. To 0.6 ml kidney cell suspension (2×10^7 cells) was added 0.4 ml either parathyroid gland (PTG) homogenate (20 mg parathyroid glands) from vitamin D-deficient chicks in the same Eagle's medium, PTG extract (10 min centrifugation, 3500 rev./min), Eagle's medium, Eagle's medium with pure BPTH or with 'trichloroacetic acid (TCA) powder' of avian PTG [12]. The relative activity of the PTG material added was estimated on the basis of cyclic AMP response [12] of a similar preparation. 26,27-^{3}H,$25(OH)D_3$ (Radiochemical Centre, Amersham, 9 mCi/mmol), 45 pmol in 10 μl ethanol, was added to the cell suspension. The vials were then gassed with O_2 for 1 min and incubated at 37°C for 3 h. Gassing was repeated at 1 h intervals. Kidney homogenates were prepared and incubated as in [3].

Following incubation, either cells or homogenates were extracted and chromatographed on Sephadex LH-20 columns [3] or were subjected to high-pressure liquid chromatography [13] for the estimation of vitamin D metabolites. Protein in kidney cells or homogenates was determined according to [14].

The results were subjected to standard statistical procedures [15].

3. Results

Kidney cells obtained from cholecalciferol deficient chicks, produced in vitro more $1\alpha,25(OH)_2D_3$ and less $24,25(OH)_2D_3$ than cells obtained from cholecalciferol-fed chicks.

Table 1
Effect of homogenates of the parathyroid gland on the production in vitro of dihydroxylated metabolites of cholecalciferol by kidney cells[a]

Dietary cholecalciferol (μg/kg)	Addition	$1\alpha,25(OH)_2D_3$ (pmol/mg protein)	$24,25(OH)_2D_3$ (pmol/mg protein)
50	None	0.79 ± 0.05[b]	3.22 ± 0.50[b]
50	PTG	1.72 ± 0.16[c]	3.06 ± 0.53[b]
0	None	3.77 ± 0.25[d]	1.77 ± 0.43[c]
0	PTG	4.56 ± 0.40[d]	1.54 ± 0.25[c]

[a] Means ± SEM obtained from 5–6 incubations
[b–d] Means designated by different letter differ significantly ($P < 0.01$)

The addition of avian PTG homogenate (20 mg gland) to kidney cells obtained from cholecalciferol-fed (tables 1,2) or deficient (table 2) chicks, enhanced the production of $1\alpha,25(OH)_2D_3$ but did not influence the production of $24,25(OH)_2D_3$. The effect of PTG homogenate on $1\alpha,25(OH)_2D_3$ production was significant ($P < 0.01$) only in cells obtained from cholecalciferol-fed chicks.

The addition of avian PTG slices, 'TCA powder' (table 2), or extracts (table 3) to kidney cells obtained from cholecalciferol-fed chicks enhanced significantly ($P < 0.01$) the production of $1\alpha,25(OH)_2D_3$ in vitro. Conversely, PTG extract did not modify $1\alpha,25(OH)_2D_3$ production by kidney homogenates. Neither PTG homogenate slices (table 2) nor extract (not given in table 3) influenced significantly ($P > 0.05$) the production of $24,25(OH)_2D_3$ by kidney cells obtained from cholecalciferol-fed chicks. 'TCA powder' slightly reduced ($P < 0.01$) $24,25(OH)D_3$ production.

The addition of 1 μg pure BPTH to kidney cells obtained from cholecalciferol-fed chicks enhanced

Table 3
The effect of parathyroid gland extract on the production in vitro of 1,25-dihydroxycholecalciferol (fmol/mg protein) by kidney cells or homogenates[a]

Incubation	Cells	Homogenates	
	180 min	15 min	180 min
None	660 ± 140[b]	19 ± 5[b]	37 ± 5[b]
PTG extract	1340 ± 30[c]	21 ± 11[b]	36 ± 6[b]

[a] Results are means ± SE of 4 or 5 determinations
[b–d] Means designated by different letters are significantly different ($P < 0.01$)

Table 2
Effect of various sources of parathyroid activity on the production of dihydroxylated metabolites of cholecalciferol by kidney cells in vitro[a]

Additive	$1\alpha,25(OH)_2D_3$ (pmol/mg protein)	$24,25(OH)_2D_3$ (pmol/mg protein)
Trial 1		
None	0.91 ± 0.11D[a]	5.10 ± 0.42[c]
BPTH (1 μg/tube)	1.36 ± 0.09[c]	3.34 ± 0.33[d]
PTG homogenate (20 mg PTG/tube)	1.56 ± 0.08	5.68 ± 0.52[d]
PTG slices (20 mg PTG/tube)	1.59 ± 0.06[c]	3.93 ± 0.34[c,d]
Trial 2		
None	0.41 ± 0.07[b]	6.67 ± 0.62[b]
PTG homogenate (20 mg PTG/tube)	0.82 ± 0.11[c]	6.42 ± 0.54[b]
PTG 'TCA powder'[b] (1 mg/tube)	0.92 ± 0.08[c]	4.17 ± 0.55[c]

[a] Means ± SEM obtained from 4–6 incubations
[b–d] Mean designated by different letters differs significantly ($P < 0.01$)

'TCA powder' was prepared from avian PTB by a procedure similar to that in [12] (A. M. et al. in preparation)

significantly the production of $1\alpha,25(OH)_2D_3$ (table 2). The response to BPTH was less pronounced than that to avian PTG material. $24,25(OH)_2D_3$ production was significantly ($P < 0.01$) reduced by BPTH (table 2).

4. Discussion

Freshly prepared kidney cells produced in vitro more $1\alpha,25(OH)_2D_3$ than whole homogenates, on the basis of tissue protein. Kidney cells, from cholecalciferol-deprived chicks, retained their ability to produce in vitro large quantities of $1\alpha,25(OH)_2D_3$, relative to cholecalciferol-fed chicks, in agreement with documented observations with homogenates [1–3]. Thus, once stimulated in vivo, the activity of 1-hydroxylase is maintained in intact kidney cells in vitro for $\geqslant$3 h. This is in accord with the relatively long half-life of the enzyme (11.3 h) as estimated (S. H. et al., in preparation) from the results in [16].

Here homogenates, slices, extract, or 'TCA powder' of avian parathyroid gland stimulated the production of $1\alpha,25(OH)_2D_3$ by kidney cells. Such an effect was observed in >15 trials carried out with kidney cells obtained from cholecalciferol-fed chicks. The increased production of $1\alpha,25(OH)_2D_3$ in response to PTG material in the various trials, was +60% to +170%. The response of the cells which had been stimulated in vivo, such as cholecalciferol-deprived birds, parathyroid material was <20%, and the effect failed to reach significance. Thus, the demonstration of a response of kidney cells to parathyroid hormone may depend on the use of animals with an appropriate nutritional history. The use of pre-stimulated animals could explain some of the earlier results which failed to indicate a response to PTG [9,10].

The response of the cells to PTG homogenate or extract was considerably lower in magnitude than that observed by in vivo stimulation (vitamin D deficiency, low calcium diet). This could be explained by the relatively short incubation time in comparison with the 3–4 days of stimulation [16] required to reach maximum enzyme activity in vivo. Nevertheless, 3 h incubation in vitro were sufficient to produce a significant response.

The response to parathyroid extract could be obtained only with intact cells (table 4) and not whole homogenates, in accord with the documented interaction of PTH with cell membrane sites [17]. Therefore, the results of this study support the concept of direct stimulation of $1\alpha,25(OH)_2D_3$ production by PTH through a mechanism requiring intact cell membranes.

Pure BPTH (table 2) at the concentration used in here resulted in a smaller response than that produced by avian PTG material, despite the lower activity of avian PTG material, based on production of cyclic AMP by kidney cells in vitro. Such a modest response was also observed in [8,11]. This would suggest some species specificity.

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References

[1] Fraser, D. R. and Kodicek, E. (1973) Nature 241, 163–166.
[2] Henry, H. L., Midgett, R. J. and Norman, A. W. (1974) J. Biol. Chem. 249, 7584–7592.
[3] Montecuccoli, G., Bar, A., Risenfeld, G. and Hurwitz, S. (1977) Comp. Biochem. Physiol. 57A, 331–334.
[4] Omdahl, J. L., Gray, R., Boyle, I. R., Knutson, J. and DeLuca, H. F. (1972) Nature 237, 63–64.
[5] Garabedian, M., Holick, M. F., DeLuca, H. F. and Boyle, I. T. (1972) Proc. Natl. Acad. Sci. USA 69, 1673–1676.
[6] Castillo, L., Tanaka, Y. and DeLuca, H. F. (1977) Arch. Biochem. Biophys. 179, 211–217.
[7] Rasmussen, H., Wong, M., Bikle, D. and Goodman, D. (1972) J. Clin. Invest. 51, 2502–2505.
[8] Trechsel, U., Bonjour, J. P. and Fleisch, H. (1979) J. Clin. Invest. 64, 206–217.
[9] Henry, H. L. (1977) Biochem. Biophys. Res. Commun. 74, 768–774.
[10] Larkins, R. G., MacAuley, S. G. and MacIntyre, I. (1974) Clin. Sci. Mol. Med. 46, 569–582.
[11] Spanoes, E., Brown, D. I. and MacIntyre, I. (1979) FEBS Lett. 105, 31–34.
[12] Aurbach, G. D. (1959) J. Biol. Chem. 234, 3179–3181.
[13] Brown, E. M., Hurwitz, S. and Aurbach, G. D. (1977) Endocrinology 100, 1969–1702.