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INFLUENCE OF PROSTAGLANDINS AND OF CYCLIC NUCLEOTIDES ON THE METABOLISM OF 25-HYDROXYVITAMIN D_3 IN PRIMARY CHICK KIDNEY CELL CULTURE [1]

Ulrich Trechsel, Carol M. Taylor, Jean-Philippe Bonjour and Herbert Fleisch

Department of Pathophysiology, University of Berne, Murtenstrasse 35, 3010 Berne, Switzerland

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SUMMARY

Prostaglandins (PG) are likely to be involved in a number of regulatory mechanisms in the kidney, which may be mediated by cyclic nucleotides. The present study describes the effects of prostaglandins and cyclic nucleotides on the hydroxylases of 25-hydroxycholecalciferol ($25(OH)D_3$) in a primary chick kidney cell culture. 3-30 nM PG E_2 produced significant increases in the $25(OH)D_3$-1-hydroxylase associated with decreases in the $25(OH)D_3$-24-hydroxylase at 6 hours but not at 1 hour. PG $F_{2\alpha}$, in concentrations between 0.3 nM and 3 μM affected the hydroxylases in a similar manner. A significant increase of 1-hydroxylase activity was observed with 0.1 mM cyclic AMP (cAMP) or dibutyryl cyclic AMP (dbcAMP) in 6 hours, but again no effect on either hydroxylase was observed when the incubation time was reduced to 1 hour. These results suggest that PG E_2 and PG $F_{2\alpha}$ might be involved in the regulation of renal $25(OH)D_3$ metabolism, and that the effects on the $25(OH)D_3$-hydroxylases might be mediated by cAMP.

INTRODUCTION

The kidney is the site (1,2,3,4) of conversion of 25-hydroxyvitamin D_3 ($25(OH)D_3$) to 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the most active form known of vitamin D_3 in the intestine (5,6) and in bone (7,8). $25(OH)D_3$ can also be metabolized in the kidney to 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) (9,10). There have been several suggestions as to the physiological role of this metabolite (11,12) but no well defined function has yet been discovered.

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The development of kidney cell culture systems by several laboratories (13,14,15,16) has provided a new tool for the investigation of long-term mechanisms involved in the regulation of $25(OH)D_3$ metabolism. In a previous report (15) we have described a primary chick kidney cell culture system capable of metabolizing ^3H-$25(OH)D_3$ to ^3H-$1,25(OH)_2D_3$ and to ^3H-$24,25(OH)_2D_3$. In this system parathyroid hormone (PTH) stimulated the $25(OH)D_3$-1-hydroxylase and reduced the 24-hydroxylase activity in 6 hours. $1,25(OH)_2D_3$ reduced the 1-hydroxylase and increased the 24-hydroxylase in 4 hours. These effects are in agreement with results obtained in vivo (17,18,19), suggesting that chick kidney cell culture represents a useful alternative to study the regulation of the renal $25(OH)D_3$-hydroxylases under in vitro conditions.

In the present study we have investigated the effects of prostaglandins (PG) and of cyclic nucleotides on the $25(OH)D_3$-hydroxylases in primary chick kidney cell culture. PG are thought to be involved in a number of regulatory mechanisms in the kidney (20) and many of the physiological effects of these compounds have been suggested to be mediated by cyclic nucleotides (21).

METHODS AND MATERIALS

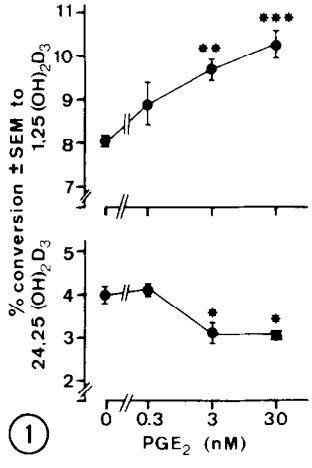
The cell cultures were produced and the $25(OH)D_3$ hydroxylases assayed as described previously (15). Briefly, kidneys of 10 day old chicks were digested with collagenase and hyaluronidase; the cells were cultured in Petri dishes, 3.5 cm in diameter, 1.2×10^6 cells, in 1.5 ml of minimum essential medium (MEM) with Earle's salt solution, antibiotics and 10 % fetal calf serum. The medium contained 1.2 mM Ca. 2 x 250 pmoles of $25(OH)D_3$ were added 24 and 48 hours after plating. Cultures were kept in a humidified CO_2-incubator, at 37°C. Confluence was obtained 3 days after plating. PG and cyclic nucleotides were addded to confluent cultures in 15 µl EtOH or distilled water. Control dishes received 15 µl solvent. Incubation with the agents was terminated by replacing the medium by 1 ml of MEM without fetal calf serum and without PG or cyclic nucleotide. The cultures were incubated with 100 pmoles of ^3H-$25(OH)D_3$ for 30 min. The reaction was stopped with methanol, tritiated vitamin D_3 metabolites were extracted into chloroform/methanol and separated on small-particle silica

using high pressure liquid chromatography (HPLC). The eluent consisted of 12.5 % isopropanol in n-hexane. Results are expressed as the percentage of the radioactivity recovered from the HPLC.

25(OH)[26,27-^{3}H]D_3 (specific activity 7.3 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, U.K. Synthetic 25(OH)D_3 was kindly donated by Hoffmann-La Roche & Co., Basel, Switzerland. PG E_2, PG $F_{2\alpha}$, cyclic AMP (cAMP), dibutyryl cyclic AMP (dbcAMP) and dibutyryl cyclic GMB (dbcGMB) were from Sigma Chemical Co., St. Louis, MO, USA. Eagle's minimum essential medium, antibiotics and fetal calf serum were from Gibco, Paisley PA3 4EP, Renfrewshire, Scotland, U.K. Collagenase (type I) and hyaluronidase (type I) were from Sigma Chemical Co. Organic solvents and all other chemicals were analytical grade.

RESULTS

Effect of prostaglandins: Figure 1 shows the effect of PG E_2 on the 25(OH)D_3-hydroxylases. A significant increase of the 1-hydroxylase associated with a decrease of the 24-hydroxylase activity was obtained with 3 nM PG E_2 in 6 hours. Separate experiments showed that 30 nM PG E_2 produced maximum effects (not shown). Figure 2 demonstrates that the effects of PG E_2 seen after 6 hours of incubation were not observed when the incubation time was reduced



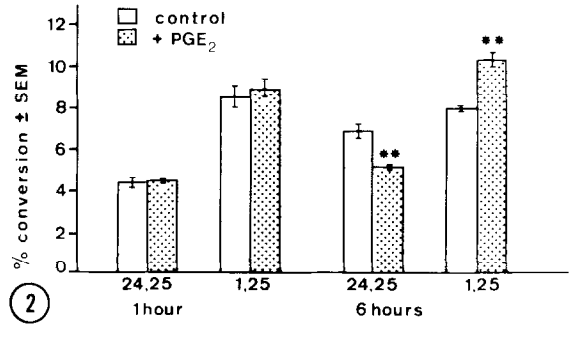


Fig. 1: Effect of PG E_2 (6 hours) on the metabolism of ^{3}H-25(OH)D_3 in primary chick kidney cell culture
Values are means ± SEM, n = 3. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ by Student's t-test for unpaired values.

Fig. 2: Evidence for a delayed response of the renal 25(OH)D_3-hydroxylases to PG E_2
Values are means ± SEM, n = 3. ** $p < 0.01$ by Student's t-test for unpaired values.

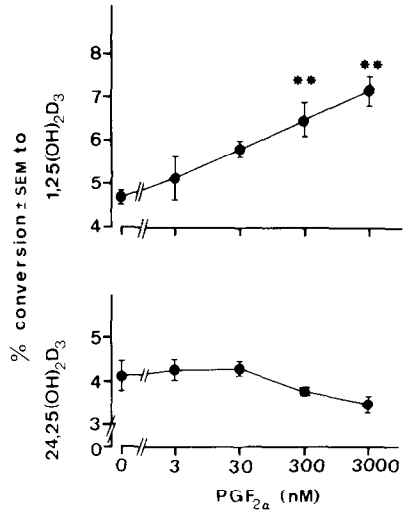


Fig. 3: Effect of PG $F_{2\alpha}$ (6 hours) on the metabolism of ^{3}H-25(OH)D_3 in primary chick kidney cell culture
Values are means ± SEM, n = 4. ** $p < 0.01$ by Student's t-test for unpaired values.

to one hour. Similarly PG $F_{2\alpha}$, in concentrations between 0.3 nM and 3 µM, produced significant changes in hydroxylase activities under idential experimental conditions (Fig. 3).

Effect of cyclic nucleotides: As shown in Table I, cAMP and dbcAMP produced a significant increase of the 25(OH)D_3-1-hydroxylase and a decrease of the 24-hydroxylase in 6 hours. In contrast dbcGMP in concentrations up to 1 mM failed to produce any effect on either hydroxylase. 1 mM cAMP added to confluent cultures for only 1 hour did not influence the 25(OH)D_3-hydroxylase activities (Fig. 4).

DISCUSSION

Our results suggest that PG E_2 and PG $F_{2\alpha}$ may act as modulators of the renal 25(OH)D_3-hydroxylases at the tissue level. A number of agents, such as bradykinin, noradrenalin and vasopressin have been shown to modulate renal PG synthesis (22). It is therefore

Table I: Effect of cyclic nucleotides on metabolism of ^{3}H-25(OH)D_3 in kidney cell culture

Cyclic nucleotide	Concentration (mM)	% conversion to ^{3}H-24,25(OH)$_2D_3$	% conversion to ^{3}H-1,25(OH)$_2D_3$
cAMP (Exp. I)	0	2.6 ± 0.2	6.7 ± 0.1
	0.1	2.5 ± 0.2	8.0 ± 0.2 **
	1	1.8 ± 0.2 *	8.5 ± 0.3 **
dbcAMP (Exp. II)	0	3.9 ± 0.2	4.7 ± 0.4
	0.01	3.4 ± 0.2	5.7 ± 0.2
	0.1	2.5 ± 0.1 **	6.7 ± 0.1 **
	1	2.3 ± 0.2 **	7.6 ± 0.4 **
dbcGMP (Exp. II)	0	3.8 ± 0.3	5.7 ± 0.2
	0.01	4.0 ± 0.1	5.1 ± 0.3
	0.1	4.2 ± 0.2	5.6 ± 0.2
	1	4.0 ± 0.2	5.7 ± 0.1

n = 3. Values are means ± SEM. * $p < 0.05$; ** $p < 0.01$ by Student's t-test for unpaired values.

conceivable that some of the physiological factors which regulate 25(OH)D_3 metabolism in vivo might act through alterations in renal PG metabolism.

As to the mechanism of action of prostaglandins at the cellular level, a large amount of evidence has been accumulated suggesting that prostaglandin-cyclic nucleotide interactions are involved in several cellular effects of prostaglandins. Especially those of

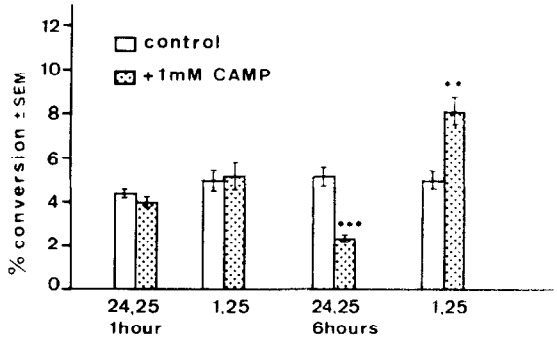


Fig. 4: Evidence for a delayed response of the renal 25(OH)D_3-hydroxylases to cAMP
Values are means ± SEM, n = 4. ** $p < 0.01$; *** $p < 0.001$ by Student's t-test for unpaired values.

the E-series have been shown to stimulate adenylate cyclase in a number of systems (21).

The effects of cAMP and dbcAMP on the 25(OH)D_3-hydroxylases in kidney cell culture are in agreement with earlier findings obtained in vivo (23) and with isolated kidney tubules in vitro (24,25), which suggested that cAMP might play a role in the regulation of the renal 25(OH)D_3 metabolism as a stimulator of the 1-hydroxylase.

Our results lend support to the hypothesis that PG E_2 and PG $F_{2\alpha}$ might stimulate the 25(OH)D_3-1-hydroxylase through an increase in cAMP. cAMP has already been suggested to be the second messenger for the stimulatory effect of PTH on the renal 25(OH)D_3-1-hydroxylase (23,24,25). Thus it is conceivable that PTH, PG E_2 and PG $F_{2\alpha}$ stimulate this enzyme through cAMP. Furthermore recent evidence obtained with isolated kidney tubules suggests an interaction between PTH and PG E_2 in elevating cAMP levels (26). Further work is needed to study whether prostaglandins might play a role in the modulation of the stimulatory effect of PTH on the 25(OH)D_3-1-hydroxylase.

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REFERENCES

1. Omdahl, J.L., and DeLuca, H.F. (1973) Physiol.Rev. 53, 327-372.
2. Norman, A.W., and H. Henry (1974) Recent Prog. Horm.Res. 30, 431-480.
3. Fraser, D.R. (1975) Proc.Nutr.Soc. 34, 139-143.
4. DeLuca, H.F., and Schnoes, H.K.(1976) Ann.Rev.Biochem. 45, 631-666.
5. Boyle, I.T., Miravet, L., Gray, R.W., Holick, M.F., and DeLuca, H.F. (1972) Endocrinology 90, 605-608.

6. Wong, R.G., Norman, A.W., Reddy, C.R., and Coburn, J.W. (1972) J.Clin.Invest. 51, 1287-1291.
7. Raisz, L.G., Trummel, C.L., Holick, M.F., and DeLuca H.F. (1972) Science 175, 768-769.
8. Reynolds, J.J., Holick, M.F., and DeLuca, H.F. (1973) Calcif. Tiss.Res.12, 295-301.
9. Holick, M.F., Schnoes, H.K., DeLuca, H.F., Gray, R.W., Boyle, I.T., and Suda, T. (1972) Biochemistry 11, 4251-4255.
10. Taylor, C.M., Mawer, E.B., Wallace, J.E., St. John, J., Cochran, M., Russell, R.G.G., and Kanis, J.A. (1978) Clin.Sci. Molec.Med. 55, 541-547.
11. Norman, A.W., and Henry, H.L. (1979) Vitamin D, Basic Research and Its Clinical Application, pp. 571-578, de Gruyter, Berlin.
12. Kanis, J.A., Cundy, J., Bartlett, M., Smith, R., Heynen, G., Warner, G.J., and Russell, R.G.G. (1978) Brit.Med.J. 1, 1382-1386.
13. Juan, D., and DeLuca, H.F. (1977) Endocrinology 101, 1184-1193.
14. Henry, H.L. (1979) J.Biol.Chem. 254, 2722-2729.
15. Trechsel, U., Bonjour, J.-P., and Fleisch, H. (1979) J.Clin. Invest. 64, 206-217.
16. Spanos, E., Barrett, D.I., Chong, K.T., and MacIntyre, I. (1978) Biochem.J. 174, 231-236.
17. Garabedian, M., Holick, M.F., DeLuca, H.F., and Boyle, I.T. (1972) Proc.Natl.Acad.Sci.USA 69, 1673-1676.
18. Fraser, D.R., and Kodicek, E. (1973) Nature (Lond.)New Biol. 241, 163-166.
19. Tanaka, Y., Lorenc, R.C., and DeLuca, H.F. (1975) Arch.Biochem. Biophys. 171, 521-526.
20. Dunn, M.J., and Hood, V.L. (1977) Am.J.Physiol. 233, F169-F184.
21. Samuelsson, B., Goldyne, M., Grandström, E., Hamberg, M., Hammarström, S., and Malmsten, C. (1978) Ann.Rev.Biochem. 47, 997-1029.
22. Danon, A., Chang, L.C., Sweetman, S.J., Nies, A.S., and Oates, J.A., (1975) Biochim.Biophys.Acta 388, 71-83.
23. Horiuchi, N., Suda, T., Takahashi, H., Shimazawa, E., and Ogata, E. (1977) Endocrinology 101, 969-974.
24. Rasmussen, H., Wong, M., Bikle, D., and Goodman, D.B.P. (1972) J.Clin.Invest. 51, 2502-2504.
25. Larkins, R.G., MacAuley, S.J., Rapoport, A. Rapoport, Martin, T.J., Tulloch, B.R., Byfield, P.G.H., Matthews, E.W., and MacIntyre, I. (1974) Clin.Sci.Molec.Med. 46, 569-582.
26. Biddulph, D.M., Currie, M.G., and Wrenn, R.W. (1979) Endocrinology 104, 1164-1171.