Biochem. J. (1983) **214**, 893–897
Printed in Great Britain

Stimulation of 1,25-dihydroxyvitamin D_3 production by 1,25-dihydroxyvitamin D_3 in the hypocalcaemic rat

Yoko TANAKA and Hector F. DELUCA
Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, WI 53706, U.S.A.

(*Received 27 April 1983/Accepted 15 June 1983*)

Serum 1,25-dihydroxyvitamin D_3 concentration and renal 25-hydroxyvitamin D 1α-hydroxylase activity were measured in rats fed various levels of calcium, phosphorus and vitamin D_3. Both calcium deprivation and phosphorus deprivation greatly increased circulating levels of 1,25-dihydroxyvitamin D_3. The circulating level of 1,25-dihydroxyvitamin D_3 in rats on a low-calcium diet increased with increasing doses of vitamin D_3, whereas it did not change in rats on a low-phosphorus diet given increasing doses of vitamin D_3. In concert with these results, the 25-hydroxyvitamin D 1α-hydroxylase activity was markedly increased by vitamin D_3 administration to rats on a low-calcium diet, whereas the same treatment of rats on a low-phosphorus diet had no effect and actually suppressed the 1α-hydroxylase in rats fed an adequate-calcium/adequate-phosphorus diet. The administration of 1,25-dihydroxyvitamin D_3 to vitamin D-deficient rats on a low-calcium diet also increased the renal 25-hydroxyvitamin D 1α-hydroxylase activity. These results demonstrate that the regulatory action of 1,25-dihydroxyvitamin D_3 on the renal 25-hydroxyvitamin D_3 1α-hydroxylase is complex and not simply a suppressant of this system.

A great deal of effort has been focused on the regulation of $1,25(OH)_2D_3$ production because this reaction forms the basis of the vitamin D endocrine system (DeLuca, 1980). Thus, hypocalcaemia (Boyle *et al.*, 1971), parathyrin (Garabedian *et al.*, 1972), phosphate depletion (Tanaka & DeLuca, 1973), sex hormones (Tanaka *et al.*, 1976) and vitamin D compounds (Larkins *et al.*, 1974; Tanaka *et al.*, 1975) are major regulating factors. $1,25(OH)_2D_3$ itself suppress 1α-hydroxylase and stimulate 24-hydroxylase (Larkins *et al.*, 1974; Tanaka *et al.*, 1975). Hypocalcaemia (Boyle *et al.*, 1971; Omdahl *et al.*, 1972) and hypophosphataemia (Baxter & DeLuca, 1976) stimulate 1α-hydroxylase, suppresses 24-hydroxylase, and causes accumulation of $1,25(OH)_2D_3$ in the serum (Boyle *et al.*, 1971; Hughes *et al.*, 1975). Exactly how these factors interact is not entirely understood. We have now found that vitamin D_3 and in particular the $1,25(OH)_2D_3$ metabolite actually stimulates the 1α-hydroxylase and causes accumulation of $1,25(OH)_2D_3$ in serum under conditions of calcium deprivation, whereas the same treatment under conditions of normal calcium and normal or low phosphorus intakes has no effect or suppresses 1α-hydroxylation and $1,25(OH)_2D_3$ accumulation.

Abbreviations used: $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3 (1,25-dihydroxycholecalciferol; calcitriol); $25(OH)D_3$, 25-hydroxyvitamin D_3 (25-hydroxycholecalciferol; calcidiol); 24-hydroxylase, 25-hydroxyvitamin D 24-hydroxylase; 1α-hydroxylase, 25-hydroxyvitamin D_3 1α-hydroxylase; 1-hydroxylase, 25-hydroxyvitamin D_3 1-hydroxylase; h.p.l.c., high-performance liquid chromatography.

Materials and methods

Vitamin D metabolites

Vitamin D_3 was purchased from Philips Roxane (New York, NY, U.S.A.), $25(OH)D_3$ was a gift of the Upjohn Company (Kalamazoo, MI, U.S.A.) and $1,25(OH)_2D_3$ was a gift of the Hoffmann–La Roche Company (Nutley, NJ, U.S.A.). $1,25(OH)_2[26,27\text{-}^3H]D_3$ (sp. radioactivity 160 Ci/mmol) was synthesized as described previously (Napoli *et al.*, 1980).

Animals

Weanling male rats were purchased from the Holtzman Company (Madison, WI, U.S.A.) and fed a vitamin D-deficient diet that contained either 0.47% calcium, 0.3% phosphorus (as a control diet with adequate calcium and adequate phosphorus, as shown in Fig. 1) (DeLuca *et al.*, 1961; Suda *et al.*,

1970), 0.02% calcium and 0.3% phosphorus (as a low-calcium diet, as shown in Fig. 2) (Garabedian *et al.*, 1972) or 0.47% calcium and 0.1% phosphorus (as a low-phosphorus diet, as shown in Fig. 3) (Tanaka & DeLuca, 1974) for 3 weeks. Where indicated rats were given various amounts of vitamin D dissolved in 0.1 ml of cottonseed oil orally each day throughout the 3 weeks. Others were given the cottonseed oil vehicle only. When $1,25(OH)_2D_3$ was given (Table 1), it was dissolved in 0.1 ml of ethanol/propylene glycol mixture (5:95, v/v) and administered subcutaneously each day for 3 weeks. Control rats received the vehicle in the same manner.

Measurement of renal 1α-hydroxylase activity

Rats were killed by decapitation and blood was collected and centrifuged to yield serum. Sera were stored at −70°C until measurement of $1,25(OH)_2D_3$. Rat kidneys were removed and placed in an ice-cold 15 mM-Tris/acetate buffer (pH 7.4 at room temperature) containing 0.19 M-sucrose, 2 mM-EGTA and 2 mM-dithiothreitol. A 5% (w/v) homogenate was prepared in the same buffer. The incubation, extraction and h.p.l.c. for measurement of the renal 1α-hydroxylase activity was carried out as described by Tanaka & DeLuca (1981).

Measurement of serum $1,25(OH)_2D_3$ concentration

Essentially, the method of Shepard *et al.* (1979) was used with slight modification utilizing chick intestinal cytosolic binding protein specific for $1,25(OH)_2D_3$.

Measurement of serum calcium and P_i concentration

Serum calcium was measured in the presence of 0.1% lanthanum chloride by means of an atomic-absorption spectrometer (Perkin–Elmer 403). Serum P_i was measured by the method described by Chen *et al.* (1956).

Results

Rats fed the vitamin D-deficient adequate-calcium/adequate-phosphorus diet developed hypocalcaemia and slight hypophosphataemia as shown in Fig. 1(*a*). These animals had increased renal 1α-hydroxylase activity as shown in Fig. 1(*b*). As expected, the vitamin D-deficient diet resulted in low circulating levels of $1,25(OH)_2D_3$ as shown in Fig. 1(*c*). A daily dose of vitamin D_3 increased serum calcium and P_i to normal (Fig. 1*a*). As expected, this treatment suppressed the renal 1α-hydroxylase activity to the detection limit of this assay method as shown in Fig. 1(*b*). A marked increase in serum $1,25(OH)_2D_3$ occurred with a daily dose of 5 i.u. (325 pmol) of vitamin D_3 with no further increase

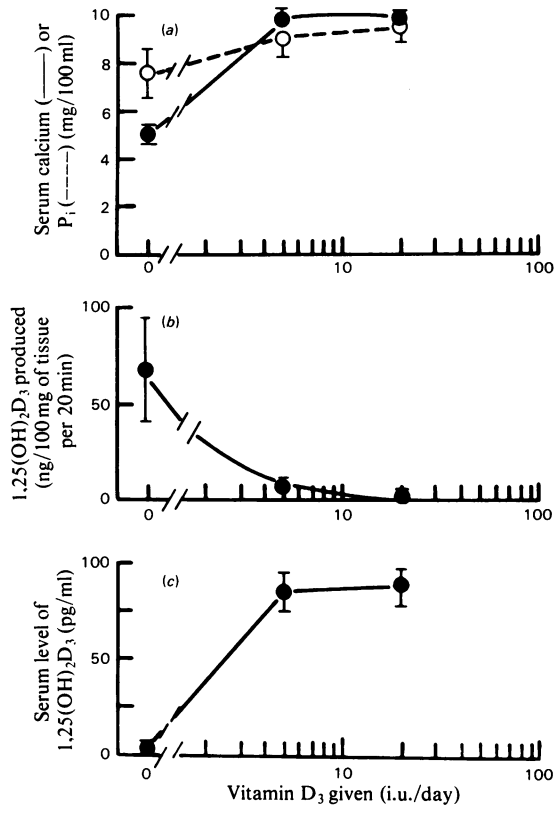

Fig. 1. *Serum calcium, serum P_i, renal 1α-hydroxylase and serum $1,25(OH)_2D_3$ concentration in rats on an adequate-calcium/adequate-phosphorus vitamin D-deficient diet and given graded doses of vitamin D_3*

Each point represents the mean value of five rats with standard deviations from the mean shown by the bars. (*a*) Serum calcium level (●) and serum P_i level (○); (*b*) renal 1α-hydroxylase activity; (*c*) serum level of $1,25(OH)_2D_3$.

apparent with a dose of 20 i.u. of vitamin D_3. These data, shown in Fig. 1, are consistent with the concept that normalized serum calcium and phosphorus concentration and elevated level of circulating $1,25(OH)_2D_3$ suppresses further production of $1,25(OH)_2D_3$. Rats on a low-calcium diet responded to the vitamin D quite differently as shown in Fig. 2. The feeding of a low-calcium diet without vitamin D caused severe hypocalcaemia as shown in Fig. 2(*a*). Serum calcium level increased with increasing vitamin D dosage, though the calcium level was still subnormal even at the 100 i.u./day dose level. Of considerable interest was the observation that the renal 1α-hydroxylase activity also increased as a function of vitamin D dose rather than being suppressed. In confirmation of the result *in vitro*, serum $1,25(OH)_2D_3$ also increased with vitamin D dosage.

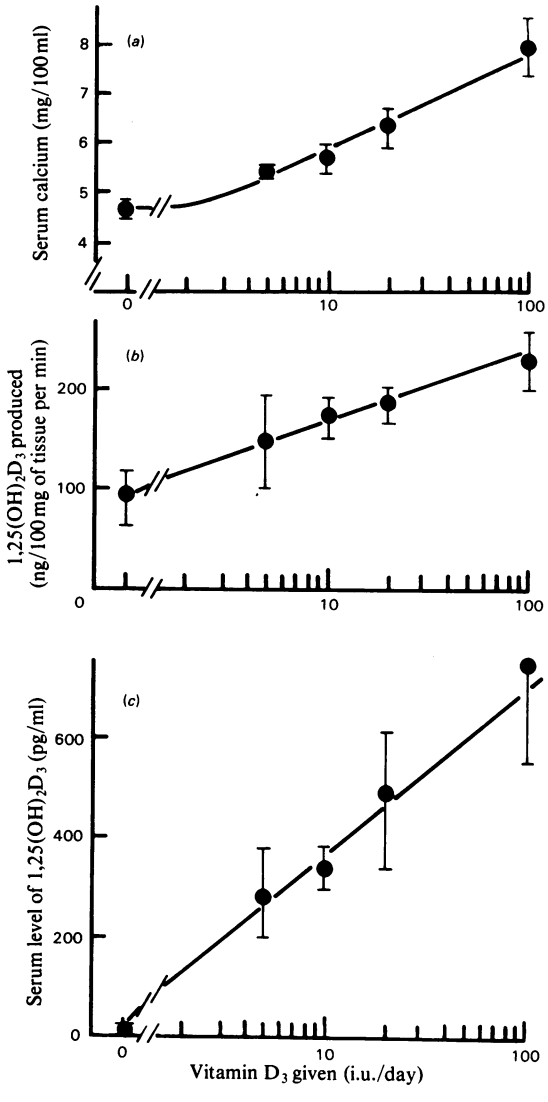

Fig. 2. *Serum calcium concentration, renal 1α-hydroxylase and serum 1,25(OH)$_2$D$_3$ concentration in rats fed a low-calcium/adequate-phosphorus vitamin D-deficient diet and graded daily doses of vitamin D$_3$*

Each point represents the mean value of five to nine rats with standard deviations from the mean shown by the bars. (*a*) Serum calcium level; (*b*) renal 1α-hydroxylase activity; (*c*) serum 1,25-(OH)$_2$D$_3$. Serum P$_i$ levels of rats in all five groups were normal.

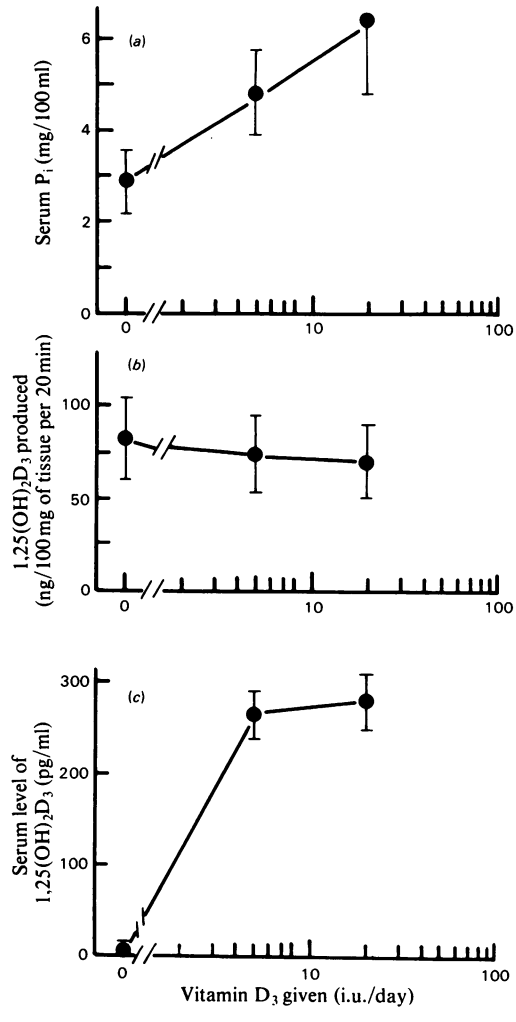

Fig. 3. *Serum P$_i$ concentration, renal 1α-hydroxylase activity and serum 1,25(OH)$_2$D$_3$ concentration in rats fed a low-phosphorus/adequate-calcium vitamin D-deficient diet and given graded daily doses of vitamin D$_3$*

Each point represents the mean value of five to six rats with standard deviations from the mean shown by the bars. (*a*) Serum P$_i$; (*b*) renal 1α-hydroxylase activity; (*c*) serum level of 1,25(OH)$_2$D$_3$. Serum calcium levels of rats in all three groups were normal.

The data in Fig. 3 indicate that administration of vitamin D$_3$ to the rats on a low-phosphorus diet did not increase the renal 1α-hydroxylase activity but it also did not suppress the 1α-hydroxylase. As shown in Fig. 3(*c*), this renal 1α-hydroxylase activity was high enough to increase substantially the circulating level of 1,25(OH)$_2$D$_3$. However, both the 1α-hydroxylase activity and serum 1,25(OH)$_2$D$_3$ levelled off despite a subnormal serum phosphorus level.

To facilitate comparison, Fig. 4 summarizes serum levels of 1,25(OH)$_2$D$_3$ in rats on either one of these three diets plotted on the same scale. It is obvious that the circulating level of 1,25(OH)$_2$D$_3$ in the rat on a low-phosphorus diet was higher than that in the rat on an adequate-calcium/adequate-

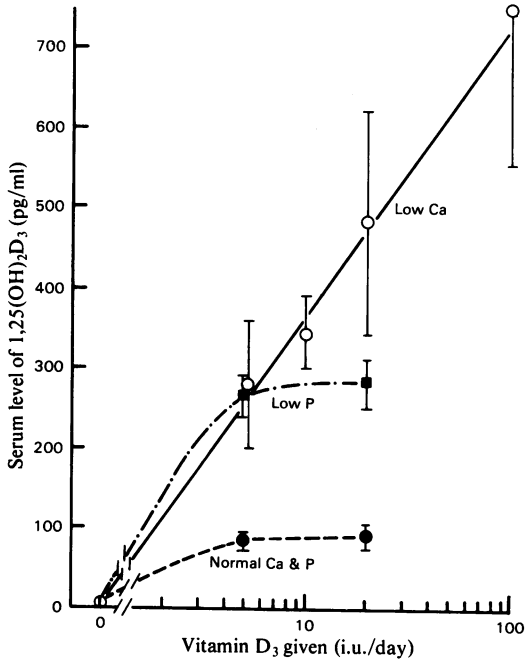

Fig. 4. *Serum 1,25(OH)$_2$D$_3$ in rats fed a low-calcium diet (low Ca), a low-phosphorus diet (low P) or an adequate-calcium/adequate-phosphorus diet (normal Ca & P) and given various amounts of vitamin D$_3$*
Figs. 1(*c*), 2(*c*) and 3(*c*) were plotted on the same scale.

Table 1. *Increase in serum calcium concentration and renal 1α-hydroxylase activity in rats on a low-calcium/adequate-phosphorus vitamin D-deficient diet and given daily doses of 1,25(OH)$_2$D$_3$*
Weanling male rats were fed a low-calcium/adequate-phosphorus vitamin D-deficient diet for 3 weeks. In the indicated group, rats were given 325 pmol of 1,25(OH)$_2$D$_3$/day dissolved in ethanol/propylene glycol (1:19, v/v) subcutaneously daily for 3 weeks. Rats in the other group received the vehicle only. All rats were killed 18 h after the last dose. The control group contained five rats; the 1,25(OH)$_2$D$_3$-dosed group contained 10 rats. Results are means ± S.D. Results for 1α-hydroxylase are significantly different at $P < 0.001$.

Compound given	Serum calcium (mg/100 ml)	1α-Hydroxylase activity (ng of 1,25(OH)$_2$D$_3$ produced/200 mg of tissue per 20 min)
Vehicle	4.4 ± 0.1	70 ± 13
1,25(OH)$_2$D$_3$	6.0 ± 1.0	164 ± 20

phosphorus diet, but the circulating levels in both cases levelled off with increasing doses of vitamin D. On the other hand, the circulating level of 1,25(OH)$_2$D$_3$ in rats on a low-calcium diet increased with increasing doses of vitamin D$_3$ at least up to 100 i.u./day.

As shown in Table 1, 1,25(OH)$_2$D$_3$ (325 pmol/day) markedly stimulated the renal 1α-hydroxylase activity in rats on a low-calcium diet, suggesting that 1,25(OH)$_2$D$_3$ itself is responsible for this action of vitamin D. The stimulation of the 1α-hydroxylase by 1,25(OH)$_2$D$_3$ is not observed under conditions of phosphate deprivation or under conditions of normal intakes of calcium and phosphorus (Y. Tanaka & H. F. DeLuca, unpublished work). Further, the 1α-hydroxylase did not increase in response to increasing 1,25(OH)$_2$D$_3$, despite a persistent hypophosphataemia in rats on a low-phosphate diet.

Discussion

The present investigation provides an additional observation in the regulation of the 1α-hydroxylase in the kidney. It had previously been demonstrated that 1,25(OH)$_2$D$_3$ administered *in vivo* or added to renal cell cultures markedly suppresses the renal 1α-hydroxylase and stimulates the 24-hydroxylase in a nuclear-mediated process (Larkins *et al.*, 1974). The present study shows that this phenomenon is not found in hypocalcaemia or calcium-deprived rats. Instead, under this circumstance vitamin D or 1,25(OH)$_2$D$_3$ actually stimulates the 1α-hydroxylase. This increase in the 1α-hydroxylase is directly related to the dose of vitamin D given. The increase in the 1α-hydroxylase activity is obviously expressed *in vivo* as well, since serum 1,25(OH)$_2$D$_3$ increases to high levels in rats on a low-calcium diet and given large does of vitamin D. Values as high as 700 pg of 1,25(OH)$_2$D$_3$/ml are observed. Thus, hypocalcaemia or parathyrin or both result in a capacity for 1,25(OH)$_2$D$_3$ to stimulate the 1α-hydroxylase. These results are consistent with the findings of Stanbury *et al.* (1981) and Papapoulos *et al.* (1980) that plasma 1,25(OH)$_2$D$_3$ levels are high in vitamin D-deficient patients being healed by administration of vitamin D. The mechanism is, of course, unknown, but it is possible that either parathyrin or nephrogenous cyclic AMP might be required for the 1,25(OH)$_2$D$_3$ stimulation of the 1α-hydroxylase. Another possibility is that high serum calcium levels are required for suppression of the 1α-hydroxylase by 1,25(OH)$_2$D$_3$. Whatever the mechanism, the increased 1α-hydroxylase that results from 1,25(OH)$_2$D$_3$ stimulation may provide a necessary and a heretofore unappreciated response to a chronic hypocalcaemic challenge.

The present paper demonstrates the complexity of the regulation of the 1α-hydroxylase by 1,25(OH)$_2$D$_3$. This compound *in vivo* can either stimulate, suppress or have no effect on this system,

depending on the calcium and phosphorus status. A simple suppression or inactivation of the 1α-hydroxylase by $1,25(OH)_2D_3$ is no longer consistent with available data. The action of $1,25(OH)_2D_3$ on this system must be coupled with calcium, parathyrin, cyclic AMP or some other factor. This observation may be of considerable importance in unravelling the subcellular events leading to the regulation of the 1α-hydroxylase.

We thank Dean Faber for his technical assistance. This work was supported by Program Project Grant no. AM-14881 from the National Institutes of Health, and by the Harry Steenbock Research Fund of the Wisconsin Alumni Research Foundation.

References

Baxter, L. A. & DeLuca, H. F. (1976) *J. Biol. Chem.* **251**, 3158–3161

Boyle, I. T., Gray, R. W. & DeLuca, H. F. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2131–2134

Chen, P. A., Jr., Toribara, T. Y. & Harnver, H. (1956) *Anal. Chem.* **28**, 1756–1758

DeLuca, H. F. (1980) *Clin. Endocrinol. Metab.* **9**, 3–26

DeLuca, H. F., Guroff, G., Steenbock, H., Reiser, S. & Mannatt, M. R. (1961) *J. Nutr.* **75**, 175–180

Garabedian, M., Holick, M. F., DeLuca, H. F. & Boyle, I. T. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1673–1676

Hughes, M. R., Brumbaugh, P. F., Haussler, M. R., Wergedal, J. E. & Baylink, D. J. (1975) *Science* **190**, 578–580

Larkins, R. G., MacAuley, S. J. & MacIntyre, I. (1974) *Nature (London)* **252**, 412–414

Napoli, J. L., Mellon, W. S., Fivizzani, M. A., Schnoes, H. K. & DeLuca, H. F. (1980) *Biochemistry* **19**, 2515–2521

Omdahl, J. L., Gray, R. W., Boyle, I. T., Knutson, J. & DeLuca, H. F. (1972) *Nature (London)* **237**, 63–64

Papapoulos, S. E., Fraher, L. J., Clemens, T. L., Gleed, J. & O'Riordan, J. L. H. (1980) *Lancet* **2**, 612–615

Shepard, R. M., Host, R. L., Hamstra, A. J. & DeLuca, H. F. (1979) *Biochem. J.* **182**, 55–69

Stanbury, S. W., Taylor, C. M., Lumb, G. A., Mawer, E. B., Berry, J., Hann, J. & Wallace, J. (1981) *Miner. Electrolyte Metab.* **5**, 212–227

Suda, T., DeLuca, H. F. & Tanaka, Y. (1970) *J. Nutr.* **100**, 1049–1052

Tanaka, Y. & DeLuca, H. F. (1973) *Arch. Biochem. Biophys.* **154**, 566–574

Tanaka, Y. & DeLuca, H. F. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1040–1044

Tanaka, Y. & DeLuca, H. F. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 196–199

Tanaka, Y., Lorenc, R. S. & DeLuca, H. F. (1975) *Arch. Biochem. Biophys.* **171**, 521–526

Tanaka, Y., Castillo, L. & DeLuca, H. F. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2701–2705