Stimulation of 1,25-dihydroxyvitamin D₃ production by 1,25-dihydroxyvitamin D₃ in the hypocalcaemic rat

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Serum 1,25-dihydroxyvitamin D₃ concentration and renal 25-hydroxyvitamin D 1α-hydroxylase activity were measured in rats fed various levels of calcium, phosphorus and vitamin D₃. Both calcium deprivation and phosphorus deprivation greatly increased circulating levels of 1,25-dihydroxyvitamin D₃. The circulating level of 1,25-dihydroxyvitamin D₃ in rats on a low-calcium diet increased with increasing doses of vitamin D₃, whereas it did not change in rats on a low-phosphorus diet given increasing doses of vitamin D₃. In concert with these results, the 25-hydroxyvitamin D 1α-hydroxylase activity was markedly increased by vitamin D₃ 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 D₃ 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 D₃ on the renal 25-hydroxyvitamin D₃ 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)₂D₃ 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)₂D₃ 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)₂D₃ 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 D₃ and in particular the 1,25(OH)₂D₃ metabolite actually stimulates the 1α-hydroxylase and causes accumulation of 1,25(OH)₂D₃ 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)₂D₃ accumulation.

Materials and methods

Vitamin D metabolites

Vitamin D₃ was purchased from Philips Roxane (New York, NY, U.S.A.), 25(OH)D₃ was a gift of the Upjohn Company (Kalamazoo, MI, U.S.A.) and 1,25(OH)₂D₃ was a gift of the Hoffmann–La Roche Company (Nutley, NJ, U.S.A.). 1,25(OH)₂[26,27-³H]D₃ (sp. radioactivity 160Ci/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)₂D₃ 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)₂D₃. Rat kidneys were removed and placed in an ice-cold 15 mM-Tris/acetate buffer (pH 7.4 at room temperature) containing 0.19 mM-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)₂D₃ 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)₂D₃.

**Measurement of serum calcium and P₁ concentration**

Serum calcium was measured in the presence of 0.1% lanthanum chloride by means of an atomic-absorption spectrometer (Perkin–Elmer 403). Serum P₁ 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)₂D₃ as shown in Fig. 1(c). A daily dose of vitamin D₃ increased serum calcium and P₁ to normal (Fig. 1a). 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)₂D₃ occurred with a daily dose of 5 i.u. (325 pmol) of vitamin D₃ with no further increase apparent with a dose of 20 i.u. of vitamin D₃. 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)₂D₃ suppresses further production of 1,25(OH)₂D₃. 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)₂D₃ also increased with vitamin D dosage.
The data in Fig. 3 indicate that administration of vitamin D₃ 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)₂D₃. However, both the 1α-hydroxylase activity and serum 1,25(OH)₂D₃ levelled off despite a subnormal serum phosphorus level.

To facilitate comparison, Fig. 4 summarizes serum levels of 1,25(OH)₂D₃ 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)₂D₃ in the rat on a low-phosphorus diet was higher than that in the rat on an adequate-calcium/adequate-
phosphorus diet, but the circulating levels in both cases leveled off with increasing doses of vitamin D. On the other hand, the circulating level of 1,25(OH)₂D₃ in rats on a low-calcium diet increased with increasing doses of vitamin D₃ at least up to 100 i.u./day.

As shown in Table 1, 1,25(OH)₂D₃ (325 pmol/day) markedly stimulated the renal 1α-hydroxylase activity in rats on a low-calcium diet, suggesting that 1,25(OH)₂D₃ itself is responsible for this action of vitamin D. The stimulation of the 1α-hydroxylase by 1,25(OH)₂D₃ 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)₂D₃, 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)₂D₃ 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)₂D₃ 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)₂D₃ increases to high levels in rats on a low-calcium diet and given large doses of vitamin D. Values as high as 700 pg of 1,25(OH)₂D₃/ml are observed. Thus, hypocalcaemia or parathyriin or both result in a capacity for 1,25(OH)₂D₃ 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)₂D₃ 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 parathyriin or nephrogenous cyclic AMP might be required for the 1,25(OH)₂D₃ 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)₂D₃. Whatever the mechanism, the increased 1α-hydroxylase that results from 1,25(OH)₂D₃ 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)₂D₃. 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)₂D₃ is no longer consistent with available data. The action of 1,25(OH)₂D₃ 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.

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References