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Low affinity of the receptor for 1α,25-dihydroxyvitamin D_3 in the marmoset, a New World monkey

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Circulating levels of 1,25-dihydroxyvitamin D are 10-fold higher in the marmoset, a New World monkey, than in man; to assess hormone receptors, we evaluated interactions of 1,25-dihydroxyvitamin D_3 with virus-transformed lympocytes. Soluble extracts of transformed lymphocytes from humans showed hormone binding with affinity and capacity similar to that of receptors for 1,25-dihydroxyvitamin D from other human tissues. However, soluble extracts of transformed lymphocytes from the marmoset showed a strikingly lower affinity for 1,25-dihydroxyvitamin D (K_d 2.2 vs 0.27 nM in marmoset vs human) and a mildly lower binding capacity (6.9 vs 16 fmol/mg protein). A defective receptor for 1,25-dihydroxyvitamin D_3 could account for resistance of target tissues to this hormone in the marmoset.

Vitamin D *Receptor* *Hormone resistance* *Osteomalacia* *Lymphocyte*

1. INTRODUCTION

1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is a seco-steroid hormone that acts in many tissues; its predominant effect at physiologic concentrations is an increased transport of calcium from the intestinal lumen to plasma. Shinki et al. [1] reported recently that serum concentrations of $1,25(OH)_2D_3$ in the marmoset (*Callithrix jacchus*) were extremely high but there was no hypercalcemia. This finding was confirmed by Adams et al. [2] in another marmoset (*Saguinus imperator*). Despite these high levels, marmosets in both studies showed signs of calcium deficiency with osteomalacia. We have examined $1,25(OH)_2D$ receptors in marmoset lymphocytes, transformed by the Epstein-Barr virus, and we now report a marked decrease in affinity of soluble cell extracts for $1,25(OH)_2D_3$.

2. MATERIALS AND METHODS

2.1. *Cell culture*

Transformed lymphocytes were generously provided by Drs Simeon Taylor, David Brandon and George Chrousos. Strains of marmoset (*S. oedipus*) B95-8 lymphocytes transformed by Epstein-Barr virus were originally obtained from Life Sciences, St. Petersburg, FL. Human lymphocytes were transformed with Epstein-Barr virus by the method of Taylor et al. [3]. The MA-250-HVP strain of stump-tailed macaque (*Macaca arctoides*) B-lymphocytes transformed by Herpesvirus papio was obtained originally from H. Rabin (Frederick Cancer Research Center, Frederick, MD). Cells were grown as suspensions in medium A (RPMI 1640 with fetal calf serum (10%), glutamine (5.8 μg/ml), and gentamycin (10 μg/ml)).

2.2. *Chemicals*

$1,25(OH)_2D_3$ was the gift of Dr M. Uskokovic (Hoffman La Roche, Nutley, NJ). $1,25[23,24(n)\text{-}^3H](OH)_2D_3$ (158 Ci/mmol) was purchased from Amersham, Arlington Heights, IL. Other chemicals were from sources reported previously [4].

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2.3. *Preparation of soluble extract from cells*

All procedures were performed at 0–4°C. Cells (approx. 3×10^8 cells per experiment) were suspended in 100 ml medium A. Medium protein was removed by resuspension in medium A without fetal calf serum at 48 and 24 h prior to harvesting; at time of harvesting they were washed in phosphate-buffered saline. Prior to sonication, the cells were resuspended in medium B (10 mM Tris-HCl, pH 7.4, 300 mM KCl, 10 mM sodium molybdate, 1.5 mM EDTA, 1.0 mM dithiothreitol, and aprotinin (500 Kallikrein Inactivator Units/ml)) at a cell concentration of 10^8/ml. The cell suspension was sonicated with three 5-s bursts from a Heat Systems W-375 sonicator in continuous mode. The sonicate was then centrifuged at $100000 \times g$ for 60 min. The supernate will be referred to as soluble extract.

2.4. *Binding of [^{3}H]1,25(OH)$_2$D$_3$ to soluble extracts from cells*

Freshly prepared soluble extract was incubated with varying concentrations of [^{3}H]1,25(OH)$_2$D$_3$ (1×10^{-10}–40×10^{-10} M) in a final volume of 0.2 ml at 4°C for 15 h. Low-affinity binding was assessed by coincubation with 1 μM 1,25(OH)$_2$D$_3$. Bound radioligand was separated from unbound with hydroxyapatite as in [4].

2.5. *Sucrose density-gradient centrifugation of radioligand bound to soluble extract*

Soluble extract was prepared and incubated with [^{3}H]1,25(OH)$_2$D$_3$ with or without 1 μM 1,25(OH)$_2$D$_3$ as indicated above. To remove unbound [^{3}H]1,25(OH)$_2$D$_3$, the soluble extract associated with [^{3}H]1,25(OH)$_2$D$_3$ was treated with dextran-coated charcoal as described [4]. The supernate was layered onto 5–20% sucrose gradients in medium B without aprotinin. This was centrifuged for 17 h at 4°C at $120000 \times g$ in a Beckman L-8 centrifuge with an SW-65 rotor. Four drop fractions were collected from the bottom of the gradient, added to 5 ml Aquasol, and assayed for ^{3}H.

Protein was determined by the method of Lowry et al. [5]. High-affinity binding was analyzed by the method of Scatchard [6]. The criterion for saturable binding was a regression analysis of bound-over-free ratio vs bound, consistent with a single class of binding sites (i.e., the slope differed from zero at the 95% level).

3. RESULTS

We evaluated the binding parameters for the interactions of [^{3}H]1,25(OH)$_2$D$_3$ with soluble extracts from transformed lymphocytes of 3 species (table 1). The average K_d (0.26 nM) and binding capacity (15 fmol/mg protein) with extract from transformed human-lymphocytes are comparable to values previously obtained for cultured dermal fibroblasts from humans (0.13 nM and 35 fmol/mg) [4].

With transformed lymphocytes, the mean value of 1,25(OH)$_2$D$_3$-binding capacity for marmoset was one half that found in cells from normal humans. The average affinity for 1,25(OH)$_2$D with soluble extract from transformed lymphocytes was 8-fold lower for marmoset vs human (table 1).

To assess whether the 1,25(OH)$_2$D$_3$-binding features were specific to the B95-8 cell line or to the marmoset species, we tested soluble extracts from macaque lymphocytes transformed by Herpesvirus papio. The binding affinity was similar to that in human cell extracts (table 1). While the comparison of receptor properties in B-lymphocytes transformed by differing viruses is not ideal, there is a strong homology in DNA sequence and

Table 1

Saturable binding of [^{3}H]1,25(OH)$_2$D$_3$ with soluble extract from virus-transformed B-lymphocytes

Donor species	K_d[a] (nM)	Capacity[a] (fmol/mg protein)
Human	0.18	18
Human	0.21	13
Human	0.08	13
Human	0.62	20
Human	0.25	13
Macaque	0.40	14
Marmoset	1.2	3.9
Marmoset	3.9	9.2
Marmoset	1.5	7.7

[a] The mean value for affinity (normalized by logarithmic transformation) and the mean value for capacity differed in the human vs in marmoset ($p < 0.05$ by Duncan's multiple range test)

The data refer to cells from 3 normal humans and one normal macaque (analyzed 1–3 times each) and from one normal marmoset (analyzed 3 times)

biological action of Epstein-Barr virus and Herpesvirus papio [7].

To characterize further the $1,25(OH)_2D_3$-binding molecules from the marmoset, we analyzed the sedimentation of bound radioligand in sucrose density gradients. The radioligand associated with extract from transformed lymphocytes of the marmoset showed a sedimentation velocity of approx. 3.5 S similar to that with extract from human transformed lymphocytes (fig.1). The difference in amplitudes of the peaks (fig.1) is attributable to a higher binding capacity in extract from human cells and to less complete binding saturation (at 4 nM [^{3}H]$1,25(OH)_2D_3$) with soluble extract from marmoset lymphocytes.

4. DISCUSSION

Receptors for $1,25(OH)_2D$ have been identified in many tissues; indistinguishable receptors have been identified in activated B lymphocytes [8]. We have confirmed that the receptor in Epstein-Barr virus transformed B-lymphocytes from the human shows properties similar to the receptor in many human tissues.

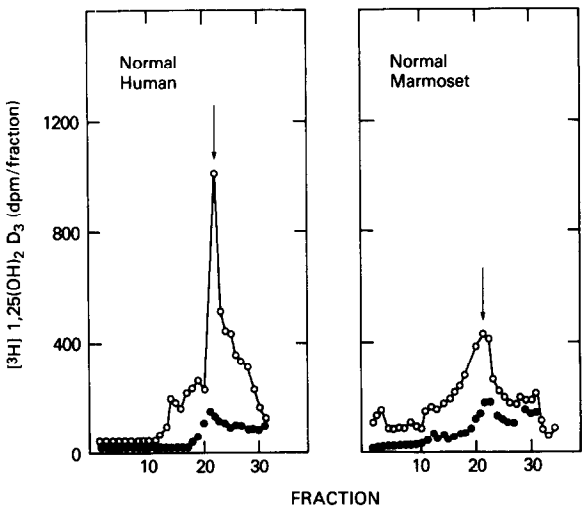

Fig.1. Sedimentation of radioligand in sucrose gradient after incubation (at 4.0 nM [^{3}H]$1,25(OH)_2D_3$) with soluble extract from transformed lymphocytes of human and of marmoset. Solid symbols represent soluble extract incubated with [^{3}H]$1,25(OH)_2D_3$ plus 1 μM $1,25(OH)_2D_3$. Radioactivity is corrected to a cytosol sample mass of 1 mg protein applied to the sucrose gradient. Arrows mark the position of [^{14}C]ovalbumin (3.7 S).

The $1,25(OH)_2D_3$-binding properties of soluble extract from transformed lymphocytes derived from the marmoset were different from those of human cells. While the capacity of saturable binding sites differed only moderately from that of human cells, the binding affinity was strikingly decreased. The 8-fold difference in binding affinity correlates well with the fact that circulating levels of $1,25(OH)_2D$ are 10-fold higher in the marmoset than in man. Thus, a difference in receptor properties may explain the unusual features of mineral metabolism in the marmoset.

Adams reported a 75–85% decrease in $1,25(OH)_2D_3$-binding capacity with intact cultured dermal fibroblasts from the marmoset (*S. imperator*) as compared to the human, somewhat more abnormal than the 57% decrease in binding capacity that we observed with transformed lymphocytes. They did not find abnormal binding affinity with marmoset cells [2]. There is precedent for discrepancies among abnormal hormone-binding parameters when studied in assays using differing cell types or cell fractions. In comparing New vs Old World monkeys, greater differences in glucocorticoid affinity and capacity were seen with soluble extract from virus transformed B-lymphocytes than with intact cells [9]. Further studies will be required to determine whether the receptors in intact vs disrupted transformed cells and in intact nontransformed cells of the marmoset are comparable.

A receptor with affinity for $1,25(OH)_2D_3$ lower than that from normal humans has been described previously only in two preliminary communications dealing with primates. Chandler et al. [10] studied interaction of $1,25(OH)_2D_3$ with LLC-MK2 cells derived from renal tissue of the rhesus (*M. mulata*), an Old World monkey. Saturable binding of radioligand was undetectable, but $1,25(OH)_2D_3$ induced 25(OH)D 24-hydroxylase enzyme in a manner suggesting mediation by a receptor with affinity 30-fold lower than in human cells. Our finding of normal affinity for $1,25(OH)_2D$ in extracts from stump-tailed macaque (*M. arctoides*) lymphocytes suggests that the LLC-MK2 cells have special features not characteristic of most cells from Old World monkeys. Castells et al. [11] described a child with hereditary resistance to

$1,25(OH)_2D$; cultured skin fibroblasts from that child showed a binding affinity for $1,25(OH)_2D_3$ 20-fold below normal. That child showed a calcemic response to high doses of $1,25(OH)_2D_3$, suggesting that affinity defects in the receptor for $1,25(OH)_2D$ could be compensated for in vivo. These observations suggest that the marmoset might serve as a valid model of hereditary resistance to $1,25(OH)_2D_3$ in humans.

New World monkeys, and the marmoset in particular, exhibit high circulating levels of glucocorticoids, mineralocorticoids, progesterone, estrogen, and $1,25(OH)_2D_3$ [1,2,9,12]. Studies with cultured cells from New World monkeys have established abnormal interactions with glucocorticoids [9,12] and $1,25(OH)_2D_3$. Although types of defect (binding affinity vs binding capacity) vary with the cell system analyzed, the overall pattern suggests that the steroid and seco-steroid hormones share a similar nuclear mechanism of action and that one disturbance during primate evolution has influenced the effector systems not only for multiple steroid hormones but also for $1,25(OH)_2D_3$, a seco-steroid hormone.

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