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The mechanism of end-organ resistance to 1α,25-dihydroxycholecalciferol in the common marmoset

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The common marmoset, a New World monkey, requires a large amount of cholecalciferol (110 i.u./day per 100 g body wt.) to maintain its normal growth. In a previous report, we demonstrated that the circulating levels of 1α,25-dihydroxycholecalciferol [1α,25$(OH)_2D_3$] in the marmosets are much higher than those in rhesus monkeys and humans, but the marmosets are not hypercalcaemic [Shinki, Shiina, Takahashi, Tanioka, Koizumi & Suda (1983) *Biochem. Biophys. Res. Commun.* **14**, 452–457]. To compare the effect of the daily intake of cholecalciferol, two rhesus monkeys were given a large amount of cholecalciferol (900 i.u./day per 100 g body wt). Their serum levels of calcium, 25-hydroxycholecalciferol and 24*R*,25-dihydroxycholecalciferol were markedly elevated, but the serum 1α,25$(OH)_2D_3$ levels remained within a range similar to those in the rhesus monkeys fed the normal diet (intake of cholecalciferol 5 i.u./day per 100 g body wt). Intestinal cytosols prepared from both monkeys contained similar 3.5S macromolecules to which 1α,25$(OH)_2D_3$ was bound specifically. However, the cytosols from the marmosets contained only one-sixth as many 1α,25$(OH)_2D_3$ receptors as those from the rhesus monkeys. Furthermore, the activity of the 1α,25$(OH)_2D_3$-receptor complex in binding to DNA–cellulose was very low in the marmosets. These results suggest that the marmoset possesses an end-organ resistance to 1α, 25$(OH)_2D_3$ and is a useful animal model for studying the mechanism of vitamin D-dependent rickets, type II.

Cholecalciferol is metabolized first in the liver to 25-hydroxycholecalciferol [25$(OH)D_3$] and then in the kidney mainly to 1α,25-dihydroxycholecalciferol [1α,25$(OH)_2D_3$] and 24*R*,25-dihydroxycholecalciferol [24*R*,25$(OH)_2D_3$] (Kodicek, 1974; DeLuca, 1974). The 1α,25$(OH)_2D_3$ is now regarded as the hormonal form of the vitamin in promoting intestinal calcium transport and bone mineral mobilization (Kodicek, 1974; DeLuca, 1974).

Recently, several lines of evidence have indicated that the 1α,25$(OH)_2D_3$ accumulates in the nuclei of target cells before exerting its biological functions (Tsai *et al.*, 1972; Zile *et al.*, 1978; Jones & Haussler, 1979). This suggests that the hormone acts in a way similar to that of other steroid hormones (Grody *et al.*, 1982). This idea was substantiated when a cytoplasmic receptor specific for 1α,25$(OH)_2D_3$ was discovered in the target tissues (Lawson & Wilson, 1974; Brumbaugh *et al.*, 1975; Kream *et al.*, 1977) and the hormone was found to be incorporated by receptor mediation into the nuclear fraction (Brumbaugh & Haussler, 1974).

In 1937, Albright *et al.* suggested the possibility that rickets arises not only from vitamin D deficiency but also from inadequate action of the vitamin. After the discovery of 1α,25$(OH)_2D_3$, it was demonstrated that patients with hereditary vitamin D-dependent rickets can be classified into two groups: those with a defect in the 1α-hydroxylation of 25$(OH)D_3$ (vitamin D-dependent rickets, type I) (Fraser *et al.*, 1973; Scriver *et al.*, 1978) and

Abbreviations used: 25$(OH)D_3$, 25-hydroxycholecalciferol; 1α, 25$(OH)_2D_3$, 1α,25-dihydroxycholecalciferol; 24*R*, 25$(OH)_2D_3$, 24*R*,25-dihydroxycholecalciferol.

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those with severe resistance to $1\alpha,25(OH)_2D_3$ (vitamin D-dependent rickets, type II) (Marx *et al.*, 1978; Tsuchiya *et al.*, 1980; Liberman *et al.*, 1980). The latter group of patients is characterized by hypocalcaemia, secondary hyperparathyroidism, osteomalacia or rickets with high circulating levels of $1\alpha,25(OH)_2D_3$. This suggests that the response to the hormone is decreased in the target tissues.

In a previous report (Shinki *et al.*, 1983), we demonstrated that the circulating levels of $1\alpha,25(OH)_2D_3$ in the common marmoset, a New World monkey, are extremely high but some of them exhibit severe osteomalacia associated with hypophosphataemia. None of the marmosets were hypercalcaemic. This led us to examine the possibility that the marmoset has an end-organ resistance to $1\alpha,25(OH)_2D_3$. In the present paper, we show that the intestinal cytosols prepared from the marmoset and the rhesus monkey, an Old World monkey, contain similar 3.5S receptor-like macromolecules, but the number of the receptors is much fewer in the marmosets than in the rhesus monkeys. Also, the activity of the $1\alpha,25(OH)_2D_3$-receptor complex in binding to DNA–cellulose is very low in the marmosets. These findings suggest that the marmoset is a useful animal model for studying the mechanism of the vitamin D-depenent rickets with severe resistance to $1\alpha,25(OH)_2D_3$.

Materials and methods

Animals

Fifteen mature common marmosets (*Callithrix jacchus*), ten males and five females, weighing approx. 300g, were fed a commercial diet containing 0.82% calcium, 0.59% phosphorus and 1,480i.u. (37μg) of cholecalciferol/kg of diet (Monkey Diet AB, Oriental Yeast Co., Tokyo, Japan) and fruit (one-eighth of an apple and one-fourth of a banana per animal every day). In addition, they were given orally 1000i.u. (25μg) of cholecalciferol twice a week. Seven young adult rhesus monkeys (*Macaca mulatta*), all females, weighing 4–6kg, were fed a commercial diet containing 0.93% calcium, 0.88% phosphorus and 2400i.u. (60μg) of cholecalciferol/kg of diet (Japan CLEA, Tokyo, Japan). Two of them were given orally 900i.u. (22.5μg) of cholecalciferol/100g body wt. every day and blood was collected from the femoral vein 1 month later.

Assay of serum concentrations of calcium, phosphorus, metabolites of cholecalciferol and parathyrin

The serum concentration of calcium was determined with an atomic absorption spectrophotometer (model 170-50A; Hitachi, Tokyo, Japan). Serum phosphorus was measured by the method of Fiske & Subbarow (1925). Serum levels of various metabolites of cholecalciferol were determined as described previously (Kano *et al.*, 1979; Shinki *et al.*, 1983). The concentrations of $25(OH)D_3$ and $24R$, $25(OH)_2D_3$ purified by Sephadex LH-20 columns and h.p.l.c. were determined by competitive protein binding assay using serum obtained from rachitic rats (Kano *et al.*, 1979). Serum $1\alpha,25(OH)_2D_3$ was also purified similarly and measured by radioreceptor assay using the Yamasa $1\alpha,25(OH)_2D_3$ receptor protein (Yamasa Shoyu Co., Chiba, Japan) (Seino *et al.*, 1982). Serum parathyrin concentrations were determined by radioimmunoassay employing a human *C*-terminal antibody (Immuno Nuclear Corp., Clearwater, MN, U.S.A.).

Preparation of intestinal cytosols

Intestines were removed from the animals and rinsed with 0.9% NaCl. The mucosa was scraped, washed with 0.9% NaCl and homogenized with 3vol. (v/w) of 0.01M-Tris buffer (0.01M-Tris–HCl, pH7.4, 0.002M-EDTA and 0.005M-dithiothreitol) containing 0.3M-KCl. The cytosols (supernatant fractions) were obtained by centrifugation (200000*g*, 1h) of the homogenates. $(NH_4)_2SO_4$-precipitated cytosols were prepared by adding 70% saturated $(NH_4)_2SO_4$ solution to yield 35% saturation, followed by centrifugation for 10min at 15000*g*. The pellets were redissolved in 0.01M-Tris buffer containing 0.15M-KCl. The protein content was determined by the method of Bradford (1976) with bovine serum albumin as the standard.

Sucrose-density-gradient analysis

Aliquots (0.3ml, 3mg of protein) of the cytosol were incubated with 2nM-$1\alpha,25(OH)_2[^3H]D_3$ in the presence or absence of excess (200nM) unlabelled $1\alpha,25(OH)_2D_3$ or $25(OH)D_3$. After incubation for 14h at 4°C, the cytosols were treated with 0.1ml of dextran-coated charcoal (dextran T 150 0.1%, charcoal 1.0%) to remove free steroid. Aliquots were then layered on to the 5–20% sucrose gradients (4.8ml) in 0.01M-Tris buffer containing 0.3M-KCl and centrifuged at 200000*g* for 20h using a Hitachi RPS 65T rotor. Fractions were collected from the top of the gradient and the radioactivity in each fraction was counted in 4ml of ACS II (Amersham). Sedimentation coefficients were estimated by comparison with protein markers (2.5S, α-chymotrypsinogen; 4.4S, bovine serum albumin).

Saturation analysis

Aliquots (0.5ml, 1mg of protein) of the cytosol were incubated for 14h at 4°C with increasing concentrations of $1\alpha,25(OH)_2[^3H]D_3$ alone or in the

presence of a 100-fold excess amount of unlabelled 1α,25(OH)$_2$D$_3$. The bound sterol was separated from the free sterol with hydroxyapatite adsorption as described by Wecksler & Norman (1979). The dissociation constant and the maximal binding of the receptors were examined by the Scatchard-plot analysis (Scatchard, 1949).

Chromatography on DNA- and DEAE-cellulose columns

DNA–cellulose was prepared from native calf thymus DNA by the method of Alberts & Herrick (1971). Aliquots (1ml, 3mg of protein) of the $(NH_4)_2SO_4$-precipitated cytosol were incubated with 2nM-1α,25(OH)$_2$[^{3}H]D$_3$ for 14h at 4°C, diluted with 0.01M-Tris buffer and applied to a DNA–cellulose column (3ml) or a DEAE-cellulose column (Whatman DE-52, 3ml) equilibrated with 0.01M-Tris buffer. The columns were washed with 20ml of the same buffer and the hormone–receptor complexes were eluted from the column with 40ml of a linear KCl gradient (0–0.5M) in 0.01M-Tris buffer. Fractions (1ml) were collected for determining KCl concentration and radioactivity. The radioactivity unbound to the column was washed out with the first 20ml of the buffer.

Measurement of DNA binding

Aliquots (0.6ml, 3mg of protein) of the cytosol were labelled with 2nM-1α,25(OH)$_2$[^{3}H]D$_3$ in the presence or absence of excess (200nM) unlabelled 1α,25(OH)$_2$D$_3$ for 14h at 4°C. Then, 0.2ml of the incubated cytosols was removed for measuring the levels of the 1α,25(OH)$_2$[^{3}H]D$_3$–receptor complexes by using the hydroxyapatite assay. To determine the activity of the 1α,25(OH)$_2$[^{3}H]D$_3$–receptor complex in binding to DNA, the incubated cytosols (0.4ml) were added to 1.6ml of a 50% slurry of DNA–cellulose suspended in 0.01M-Tris buffer, vigorously mixed and incubated for 30min at 4°C. DNA–cellulose was collected by centrifugation at 2000*g* for 10min and washed three times with 5ml of 0.01M-Tris buffer containing 0.5% Triton X-100. The 1α,25(OH)$_2$[^{3}H]D$_3$–receptor complex was extracted twice with 1ml of 0.01M-Tris buffer containing 0.3M-KCl and the radioactivity was counted in 8ml of ACS II. The amount of the 1α,25(OH)$_2$[^{3}H]D$_3$–receptor complex was calculated by subtracting nonspecific binding [in the presence of 200nM-1α,25(OH)$_2$D$_3$] from total binding. Also, the ratio of the 1α,25(OH)$_2$[^{3}H]D$_3$–receptor complex bound to DNA–cellulose to the 1α, 25(OH)$_2$[^{3}H]D$_3$–receptor complex added was measured.

Chemicals

Radioactive 1α,25(OH)$_2$[23,24-^{3}H]D$_3$ (85Ci/mmol) was obtained from Amersham International, Amersham, Bucks., U.K. Crystalline 25(OH)D$_3$ was purchased from Phillips Duphar Co., Amsterdam, The Netherlands. Crystalline 1α,25(OH)$_2$D$_3$ was kindly donated by Dr. I. Matsunaga, Chugai Pharmaceutical Co., Tokyo, Japan. Native calf thymus DNA was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Results

The marmoset requires large amounts of vitamin D to maintain its normal growth (Deinhardt, 1970). The daily cholecalciferol intake of the marmosets used in this study was 22 times higher than that of the rhesus monkeys maintained on a normal diet. To examine the effect of the daily intake of cholecalciferol, two of the rhesus monkeys were administered 900i.u. of cholecalciferol/100g body wt. every day for 1 month. Table 1 shows the serum concentrations of calcium, phosphorus, various metabolites of cholecalciferol and parathyrin in marmosets, and in rhesus monkeys fed a normal diet or ones supplemented with a large amount of cholecalciferol. When the large amount of cholecalciferol was administered to two of the rhesus monkeys, their serum 25(OH)D$_3$ and 24*R*,25(OH)$_2$D$_3$ increased strikingly. An apparent hypercalcaemia was seen in these two monkeys, but the serum levels of 1α,25(OH)$_2$D$_3$ were strictly regulated within a range similar to those in the rhesus monkeys fed the normal diet. In the marmosets, the circulating levels of 25(OH)D$_3$ were about 10 times higher than those in the rhesus monkeys fed the normal diet, because of their high daily intake of cholecalciferol. Serum 24*R*,25(OH)$_2$D$_3$ levels in the marmosets were only 3–4 times higher than those in the rhesus monkeys fed the normal diet, but the serum 1α,25(OH)$_2$D$_3$ levels in the marmosets were much higher than those in both groups of the rhesus monkeys. In spite of the extremely high serum levels of 1α,25(OH)$_2$D$_3$, the marmosets were hypocalcaemic. The serum levels of parathyrin were about 8 times higher in the marmosets than in the rhesus monkeys fed the normal diet. In the rhesus primates supplemented with the large amount of cholecalciferol, serum parathyrin was not detected. No significant difference in serum phosphorus was seen between the marmosets and rhesus monkeys.

We next examined the physical and biochemical properties of intestinal 1α,25(OH)$_2$D$_3$ receptors from the marmosets and rhesus monkeys. Fig. 1 shows sucrose-density-gradient profiles of the 1α,25(OH)$_2$D$_3$ binding to the cytosols prepared from the intestinal tissues of the rhesus monkeys and marmosets. A macromolecular component which binds to 1α,25(OH)$_2$[^{3}H]D$_3$ and sediments

Table 1. *Serum concentrations of calcium, phosphorus, metabolites of cholecalciferol and parathyrin in marmosets and in rhesus monkeys fed a normal diet or diets supplemented with a large amount of cholecalciferol*

Daily cholecalciferol intake was calculated on the basis of the mean daily food intake by the rhesus monkeys (100g) and marmosets (20g). Data except those on rhesus monkeys supplemented with a large amount of cholecalciferol are means ± S.E.M. for five rhesus monkeys or 15 marmosets. Data on rhesus monkeys given 900 i.u. of cholecalciferol/day per 100g body wt. are shown individually. Significance of difference from rhesus monkeys fed the normal diet (by Student's *t* test): † $P < 0.05$, ‡ $P < 0.005$.

Daily cholecalciferol intake	Rhesus monkey		Marmoset
(i.u./day per 100g body weight) ...	5	900	110
No. of animals ...	5	2	15
Calcium (mg/100ml)	9.4±0.3	11.5 12.7	8.4±0.2†
Phosphorus (mg/100 ml)	4.3±0.5	3.9 5.7	4.5±0.2
25(OH)D_3 (ng/ml)	50±4	1352 1651	478±108†
24*R*,25(OH)$_2D_3$ (ng/ml)	2.6±0.5	56.4 46.7	9.2±1.6†
1α,25(OH)$_2D_3$ (pg/ml)	95±17	73 74	491±93†
Parathyrin (ng/ml)	0.23±0.06	<0.1 <0.1	1.75±0.20‡

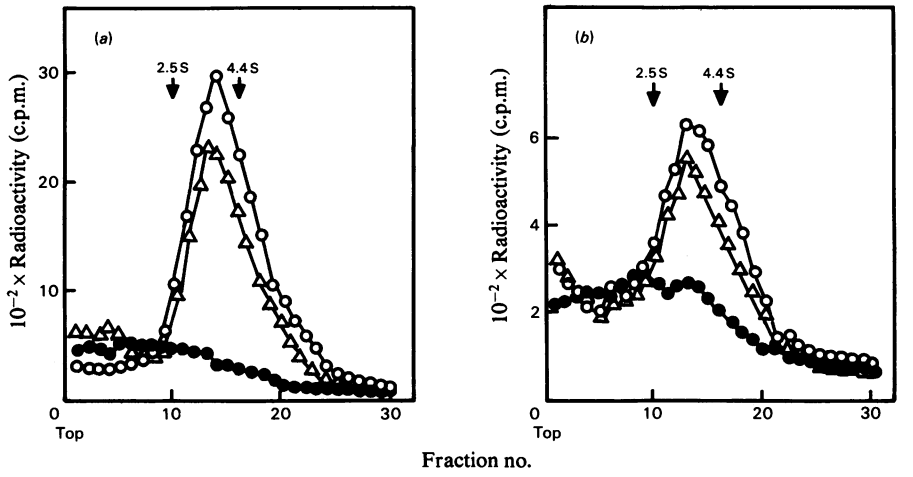

Fig. 1. *Sucrose-density-gradient analysis of 1α,25(OH)$_2$[^{3}H]D_3 binding by intestinal cytosols prepared from rhesus monkeys (a) and marmosets (b)*

Aliquots (0.3ml, 3mg of protein) of the cytosol were incubated with 2nM-1α,25(OH)$_2$[^{3}H]D_3 in the absence (○) or presence of unlabelled 200nM-1α,25(OH)$_2D_3$ (●) or 25(OH)D_3 (△) for 14h at 4°C. Arrows indicate the sedimentation positions of α-chymotrypsinogen (2.5S) and bovine serum albumin (4.4S).

at 3.5S was detected in both cytosols, but the radioactivity of the 3.5S peak was much greater in the rhesus monkeys than in the marmosets. In both primates, association of 1α,25(OH)$_2$[^{3}H]D_3 with the 3.5S macromolecule was completely abolished by adding a 100-fold excess of 1α,25(OH)$_2D_3$ but was only partially displaced by the same concentration of unlabelled 25(OH)D_3.

Fig. 2 represents the concentration-dependent 1α,25(OH)$_2$[^{3}H]D_3 binding to both cytosols. Saturation of the specific binding occurred at a concentration as low as 1.0nM-1α,25(OH)$_2D_3$. Scatchard-plot analysis of the specific binding yielded similar dissociation constants (rhesus, 0.22nM; marmoset, 0.30nM). There was approx. only one-sixth as many 1α,25(OH)$_2D_3$ receptors in the marmosets (28fmol/mg of protein) as in the rhesus monkeys (158fmol/mg of protein).

Fig. 3(*a*) illustrates the chromatographic profiles of the 1α,25(OH)$_2D_3$ binding in the intes-

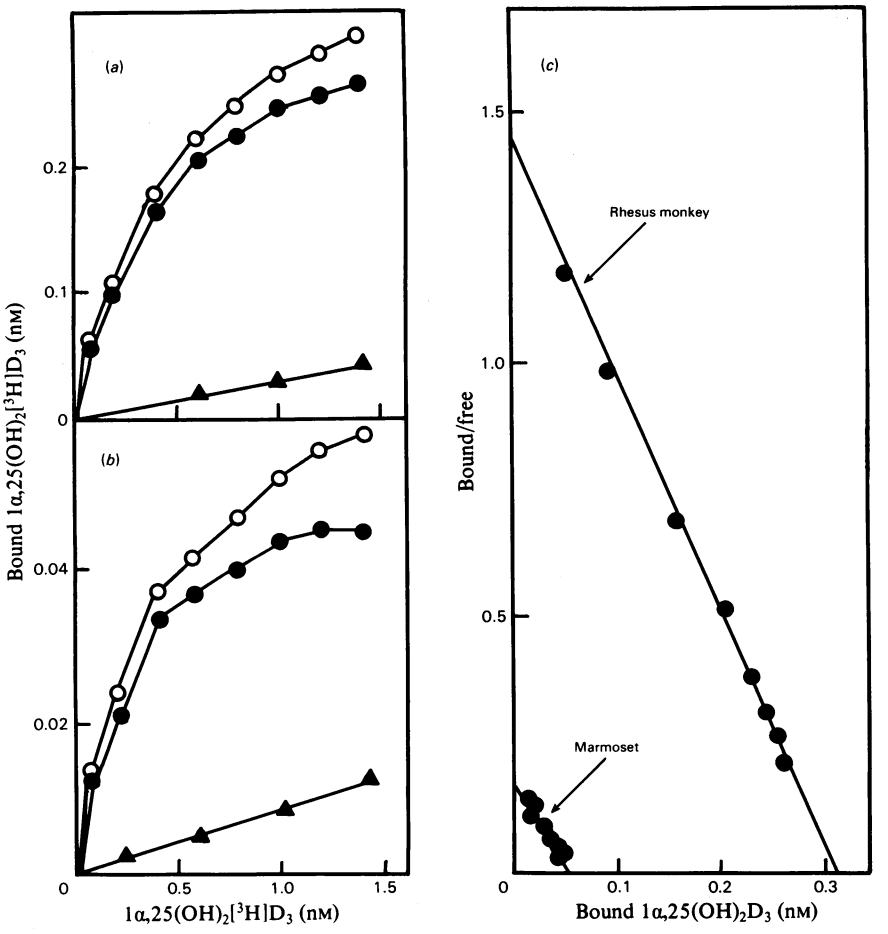

Fig. 2. *Saturation analysis of 1α,25(OH)$_2$[^{3}H]D$_3$ binding in the intestinal cytosols of rhesus monkeys (a) and marmosets (b)* Aliquots (0.5 ml, 1 mg of protein) of the cytosol were incubated with graded concentrations of 1α,25(OH)$_2$[^{3}H]D$_3$ for 14h at 4°C in the presence (nonspecific binding) or absence (total binding) of a 100-fold excess of unlabelled 1α,25(OH)$_2$D$_3$. Specific binding (●) was determined by subtracting nonspecific binding (▲) from total binding (○). (*c*) Scatchard-plot analysis of the specific binding data shown in (*a*) and (*b*).

tinal cytosols from the rhesus monkeys. The 1α,25(OH)$_2$[^{3}H]D$_3$–receptor complex was adsorbed to DNA–cellulose and eluted with a KCl linear gradient at 0.18M-KCl. In contrast, the complex from the marmosets was only slightly retained by DNA–cellulose (Figs. 3*b*, 3*c* and 3*d*). In three different preparations, a small radioactive peak was detected only in the last trial (Fig. 3*d*). 1α,25(OH)$_2$[^{3}H]D$_3$-binding macromolecules both from the rhesus monkeys and marmosets were adsorbed to DEAE-cellulose and eluted as a single peak with a linear KCl gradient at 0.13M-KCl (Fig. 4), indicating that the 1α,25(OH)$_2$[^{3}H]D$_3$–receptor complex of the marmosets markedly reduced the ability to bind to DNA–cellulose but not to DEAE–cellulose. We therefore compared the ratio of the 1α,25(OH)$_2$D$_3$–receptor complex interacting with DNA with the 1α,25(OH)$_2$D$_3$–receptor complex added in the two primates. The total amount of the 1α,25(OH)$_2$[^{3}H]D$_3$–receptor complex in the marmosets was one-eighth that in the rhesus monkeys (Table 2). These data are similar to those obtained from the Scatchard-plot analysis shown in Fig. 2. About 40% of the 1α,25(OH)$_2$-[^{3}H]D$_3$–receptor complex added was bound to DNA–cellulose in the rhesus monkeys, whereas only 4% of the hormone–receptor complex added was bound to DNA–cellulose in the marmosets (Table 2). When the cytosol of the marmosets was prepared in the presence of 3mM-phenylmethanesulphonyl fluoride, a proteolytic enzyme inhibitor, neither the activity of the cytosol in binding to 1α,25(OH)$_2$[^{3}H]D$_3$ nor that of the hormone–receptor complex in binding to DNA–cellulose was affected by the proteolytic enzyme inhibitor (results not shown).

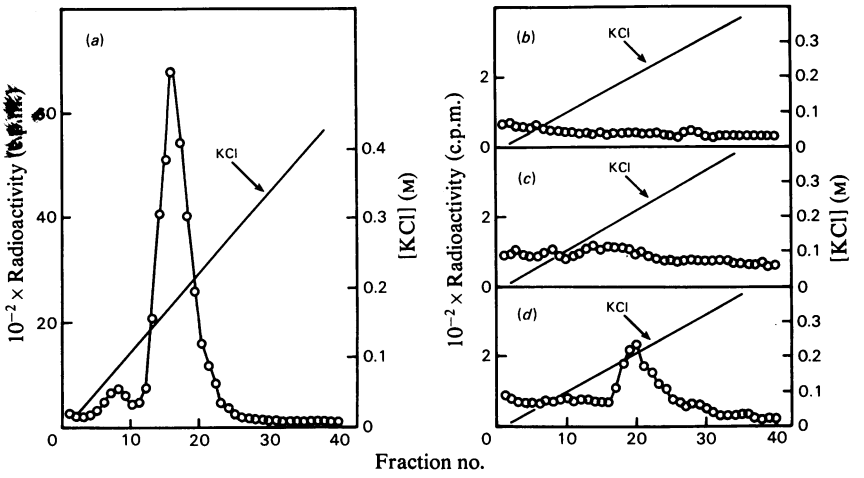

Fig. 3. *DNA–cellulose column chromatography of 1α,25(OH)$_2$[^{3}H]D$_3$ binding in (NH$_4$)$_2$SO$_4$-precipitated cytosols of rhesus monkeys (a) and marmosets (b, c and d)*

The cytosols (1 ml, 3 mg of protein) incubated with 2 nM-1α,25(OH)$_2$[^{3}H]D$_3$ for 14 h at 4°C were applied to DNA–cellulose columns. The columns were washed with 20 ml of 0.01 M-Tris buffer and the hormone-receptor complexes were eluted with a linear salt gradient as described in the Materials and methods section. The radioactivity unbound to the column was washed out with the first 20 ml of the buffer. In the marmosets, DNA–cellulose column chromatography was performed in three different preparations (*b*, *c* and *d*).

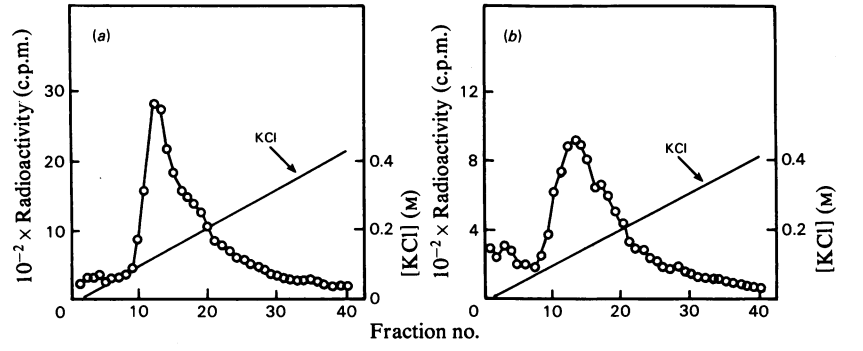

Fig. 4. *DEAE-cellulose column chromatography of 1α,25(OH)$_2$[^{3}H]D$_3$ binding in (NH$_4$)$_2$SO$_4$-precipitated cytosols of rhesus monkeys (a)* and marmosets (*b*)

The incubation of cytosols with 1α,25(OH)$_2$[^{3}H]D$_3$ and DEAE-cellulose column chromatography were performed as described in the legend to Fig. 3.

Table 2. *Activity of 1α,25(OH)$_2$[^{3}H]D$_3$–receptor complexes in intestinal cytosols of rhesus monkeys and marmosets in binding to DNA–cellulose*

Cytosols (0.6 ml, 3 mg of protein) were incubated with 2 nM-1α,25(OH)$_2$[^{3}H]D$_3$ in the presence or absence of a 100-fold excess of unlabelled 1α,25(OH)$_2$D$_3$ for 14 h at 4°C. An aliquot (0.2 ml) was used to determine the amount of the 1α,25(OH)$_2$D$_3$–receptor complex and the rest (0.4 ml) for the measurement of the binding of the complex to DNA–cellulose as described in the Materials and methods section. Data are means ± S.E.M. for three rhesus monkeys or five marmosets. Significance of difference from rhesus monkeys (by Student's *t* test): * $P < 0.005$.

Species	1α,25(OH)$_2$[^{3}H]D$_3$–receptor complexes added (fmol/0.4 ml of cytosol)	1α,25(OH)$_2$[^{3}H]D$_3$–receptor complexes bound to DNA–cellulose	
		fmol	%
Rhesus monkey	234.8 ± 32.2	110.4 ± 38.7	43.8 ± 11.7
Marmoset	33.1 ± 4.3*	1.5 ± 0.4	4.3 ± 1.1*

Discussion

In the previous report (Shinki *et al.*, 1983), we showed that marmosets are not hypercalcaemic in spite of the extremely high circulating levels of $1\alpha,25(OH)_2D_3$. In two marmosets which had suffered bone fractures, serum levels of the vitamin were particularly elevated but rachitic bone changes were found by histological examination (Suda *et al.*, 1985). In contrast, rhesus monkeys fed a vitamin D-deficient diet containing adequate calcium and phosphorus for more than 5 years do not show any rachitic signs (T. Suda, N. Takahashi, T. Shinki, A. Yamaguchi & Y. Tanioka, unpublished results). Thus, it is very likely that the marmoset requires much larger amounts of cholecalciferol to maintain its normal growth compared with the rhesus monkeys.

To examine the effect of the daily intake of cholecalciferol in the Old and New World primates, two rhesus monkeys were given a large amount of cholecalciferol for 1 month. Their serum levels of $25(OH)D_3$ and $24R,25(OH)_2D_3$ increased markedly, but those of $1\alpha,25(OH)_2D_3$ remained unchanged. Thus, the renal biosynthesis of $1\alpha,25(OH)_2D_3$ appeared to be strictly regulated in the rhesus monkeys in spite of the high intake of cholecalciferol. We also examined $25(OH)D_3$ metabolism using kidney homogenates prepared from both primates. In the rhesus monkeys fed a normal diet 24-hydroxylase was the major enzyme, whereas in the marmosets 1α-hydroxylase activity was particularly elevated in spite of the high intake of the vitamin (T. Suda, N. Takahashi, T. Shinki, A. Yamaguchi & Y. Tanioka, unpublished results).

It is known that in higher vertebrates, renal biosynthesis of $1\alpha,25(OH)_2D_3$ is tightly regulated by the circulating levels of calcium (Boyle *et al.*, 1971), phosphorus (Tanaka & DeLuca, 1973), parathyrin (Garabedian *et al.*, 1972), oestrogen (Tanaka *et al.*, 1976; Baksi & Kenny, 1977), $1\alpha,25(OH)_2D_3$ (Tanaka & DeLuca, 1974), and other peptide and steroid hormones (Fraser, 1980). Some of these regulatory factors may be involved in the increase in serum $1\alpha,25(OH)_2D_3$ levels in the marmosets. Serum parathyrin levels in the marmosets were about 8 times higher than those in the rhesus monkeys. This is secondary hyperparathyroidism due to hypocalcaemia, which may stimulate renal biosynthesis of $1\alpha,25(OH)_2D_3$. Another possible cause of the high circulating levels of $1\alpha,25(OH)_2D_3$ appears to be a defect in the mechanism by which $1\alpha,25(OH)_2D_3$ controls renal $25(OH)D_3$ metabolism. $1\alpha,25(OH)_2D_3$ is the major inhibitory factor for the 1α-hydroxylase activity (Tanaka & DeLuca, 1974; Fraser, 1980). Since the intestinal cytosol receptor system for $1\alpha,25(OH)_2D_3$ has some defects as described below, it is likely that the renal 1α-hydroxylation of $25(OH)D_3$ proceeds without any feedback control by $1\alpha,25(OH)_2D_3$.

It is of great interest that the marmoset is not hypercalcaemic in spite of the extremely high circulating levels of $1\alpha,25(OH)_2D_3$. The intestinal cytosols prepared from the marmosets also contained a 3.5S receptor-like macromolecule for $1\alpha,25(OH)_2D_3$ which was very similar to that from the rhesus monkeys, but it was only one-sixth as abundant in the marmosets as in the rhesus monkeys. Although the $1\alpha,25(OH)_2[^3H]D_3$–receptor complex of either the marmosets or rhesus monkeys was similarly adsorbed to DEAE—cellulose and eluted from the column with a similar concentration of KCl, in the marmosets the activity of the complex in binding to DNA–cellulose was very low (Table 2). Therefore, it appears that the DNA-binding ability of the $1\alpha,25(OH)_2D_3$ receptor system is inherently suppressed in the marmosets. Severe resistance to $1\alpha,25(OH)_2D_3$ in the marmosets appears to be due to a receptor-mediated decrease in the sensitivity to the hormone.

In our experiments, cytosols were incubated with $1\alpha,25(OH)_2D_3$ at 4°C, at which temperature only unoccupied receptors for $1\alpha,25(OH)_2D_3$ can be measured (Hunziker *et al.*, 1980). Since occupied $1\alpha,25(OH)_2D_3$ receptors are also important in inducing physiological response, we measured the level of the endogenously occupied receptor in the marmosets by the methods of either Hunziker *et al.* (1980) or Massaro *et al.* (1983). Unfortunately, neither attempt was successful because of the extremely low levels of the receptor and because of the reduced activity of the $1\alpha,25(OH)_2D_3$–receptor complex in binding to DNA. Massaro *et al.* (1983) separately measured occupied and unoccupied $1\alpha,25(OH)_2D_3$-binding sites in intestinal cytosols prepared from rats with various levels of plasma $1\alpha,25(OH)_2D_3$. They reported that even in rats with more than 500pg of $1\alpha,25(OH)_2D_3$/ml of plasma, occupied binding sites were only 19% of the total binding activity. Therefore, occupied receptors appear to be a small fraction of the total receptors in the marmosets whose serum $1\alpha,25(OH)_2D_3$ levels are extremely elevated.

The marmoset closely resembles vitamin D-dependent rickets, type II, with regard to hypocalcaemia, secondary hyperparathyroidism, osteomalacia or rickets with normal or high vitamin D intake, and elevated serum levels of $1\alpha,25(OH)_2D_3$. Eil *et al.* (1981) first reported that skin fibroblasts isolated from vitamin D-dependent patients are defective in nuclear uptake of $1\alpha,25(OH)_2[^3H]D_3$. Subsequently, Feldman *et al.* (1982) and Clemens *et al.* (1983) independently demonstrated that the cytosols prepared from

fibroblasts isolated from a patient with severe vitamin D-dependent rickets, type II, contain no receptor for $1\alpha,25(OH)_2D_3$. More recently, Liberman *et al.* (1983) examined the interaction of $1\alpha,25(OH)_2D_3$ with skin fibroblasts from six kindreds with this disease and reported four cases: (*a*) cytosol binding and nuclear uptake both unmeasurable; (*b*) decreased capacity and normal affinity of both cytosol binding and nuclear uptake; (*c*) normal capacity and normal affinity of cytosol binding but unmeasurable nuclear uptake; and (*d*) normal capacity and normal affinity of both cytosol binding and nuclear uptake. The decrease in the number of $1\alpha,25(OH)_2D_3$ receptors and the diminished activity of the $1\alpha,25(OH)_2D_3$–receptor complex in binding to DNA in the marmosets suggest that this primate resembles cases (*b*) and (*c*).

Other New World primates, including common marmoset, have been also reported to have extremely high plasma concentrations of progesterone, oestrogen, androgen and cortisol (Preslock *et al.*, 1973; Wolf *et al.*, 1977; Yamamoto *et al.*, 1977; Abbott & Hearn, 1978; Hearn *et al.*, 1978; Wilson *et al.*, 1978; Bonney *et al.*, 1979). Recently, Chrousos *et al.* (1982, 1984) reported that the number of progesterone receptors in the uterine cytosol of the New World squirrel monkey is only one-eighth as many as that of the Old World cynomolgus monkeys, though the affinity of the receptor for progesterone was similar in both species. These findings, together with our present study, suggest that the New World primates possess in common a distinctive receptor system for steroid hormones including $1\alpha,25(OH)_2D_3$, and that the marmoset is a useful animal model for studying the mechanism of action of $1\alpha,25(OH)_2D_3$ in the target tissues.

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