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EFFECT OF ACTIVE VITAMIN D_3 ON THE LEVELS OF NADPH-DEPENDENT CYTOSOLIC 3,5,3'-TRIIODO-L-THYRONINE-BINDING PROTEIN

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SUMMARY: Effect of 1α-OH-vitamin D_3(1α-OH-D_3) and 1,25-$(OH)_2$-vitamin D_3 (1,25-$(OH)_2$-dihydroxycholecalciferol)(1,25-$(OH)_2$-D_3) on the levels of NADPH-dependent cytosolic 3,5,3'-triiodo-L-thyronine(T_3)-binding protein (CTBP) was studied in rats and cultured dRLh cells. Deprivation of rats from vitamin D decreased the activity of cytosolic NADPH-dependent T_3 binding in rat kidney and liver. The decrease was restored by administration of 1α-OH-D_3(0.2 μg/kg). The activity of cytosolic NADPH-dependent T_3 binding was increased in the dRLh cells by addition of 1,25-$(OH)_2$-D_3 to the culture medium. The maximal binding capacity(MBC) was increased by 1,25-$(OH)_2$-D_3 without changes in the affinity constant. These results suggested that active vitamin D_3 plays an important role in the regulation of cellular T_3 translocation through increasing the binding capacity of NADPH-dependent cytosolic T_3-binding protein.

In our previous study, we demonstrated that serum concentration of thyrotropin and response of TSH to thyrotropin-releasing hormone(TRH) was lower in patients or experimental animals with high plasma concentration of active vitamin D_3 than those with low plasma concentration of active vitamin D_3(1). Although the active vitamin D_3 was speculated to control action of 3,5,3'-triiodo-L-thyronine(T_3) in its negative feedback regulation, we could not evaluate whether the decrease of TSH concentration was caused from hypercalcemia or from direct action of active vitamin D_3. On the other hand, we found the high affinity NADPH-dependent cytosolic 3,5,3'-triiodo-L-thyronine(T_3) binding-protein (CTBP) in rat kidney(2,3) which plays a role in the regulation of intracellular T_3 translocation(4,5). Further, we observed that the activity of the

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protein was reciprocally controlled by oxidized- and reduced-nicotinamide adenine dinucleotide phosphates(6). NADPH-activated form plays to reserve the cytosolic T_3, whereas NADP-activated form actively transports the hormone from cytoplasm to nuclear receptors(5-6). These findings suggested that the content of this protein is an important factor to regulate the action of thyroid hormone(7). In this study we investigated the effect of active vitamin D_3 on the CTBP in rats and cultured cells.

MATERIALS AND METHODS

Preparation of Experimental Animals: Fifty-g male Wistar rats were divided into two groups; the first group(A)(7 rats) was treated with regular diet, and the other(18 rats) with vitamin D deficient diet(Nicchiku Pharmaceutical Inc., Tokyo, Japan) which was prepared according to the method of Suda et al.(8). The latter group was further divided into three groups(B-D) 3 weeks after the beginning of the treatment. Group B received(i.p.) saline at 0 and 24 hr. The Group C was administered(i.p) 1α-OH-vitamin D_3(1α-OH-D_3)(Chugai Pharmaceutical Co., Tokyo, Japan)(0.2 μg/kg) at 0 hr. The group D was administered 1α-OH-D_3(0.2 μg/kg) at 0 and 24 hr. The cytosol fraction of kidney and liver was prepared 12(group C) or 24 hrs(group D) after the last injection of 1α-OH-D_3 or saline(B). The serum calcium concentration of these animals was measured with the spectrometric method as previously described(1).

Cell Culture: Rat hepatoma-derived cells(dRLh cells)(established by Sato, J.)(obtained from Japanese Cancer Research Resources Bank, Tokyo, Japan) were cultured in HAM'S F-12K(Flow Laboratories, Irvine, Scotland) medium containing 10% fetal calf serum at 37°C in the humidified atmosphere of 5% CO_2-95% air. 1,25-(OH)$_2$-vitamin D_3(1,25-(OH)$_2$-D_3)(Chugai Pharmaceutical Co., Tokyo, Japan) was dissolved in 99.9% ethanol, and the cells were incubated in the absence or presence of 1,25-(OH)$_2$-D_3 for 12 or 24 hrs. The final concentration of ethanol was 0.04 % in the culture medium. Control cultures received the same amount of ethanol as treated cultures. In several experiments the cells were incubated in the presence of 0.1 μg/ml actinomycin D(Sigma). After incubation, cells were detached by a brief incubation with phosphate buffered-saline(PBS), pH 7.4, containing 0.02% EDTA and 0.25% trypsin (Flow Laboratories), and cytosol fraction was prepared.

Preparation of Cytosol Fraction: The cytosol fraction from rat kidney and liver was prepared as previously described(3-5). The cytosol of each tissue was incubated with 10 % charcoal(Sargent-Welch Scientific Co., Skokie, IL) at 0°C for 30 min in order to remove pyridine nucleotides and T_3 which contaminated the cytosol. This treatment reduced the concentration of these materials to less than 0.1 % of the original cytosol(2). The cytosol from cells was prepared as follows. After incubation, cells(3.6×10^7 cells) were washed with phosphate-buffered saline(PBS), pH 7.4, for 3 times and homogenized in 1.0 ml of 10 mM Tris-HCl, pH 7.4, containing 0.32 M sucrose and 0.5 mM EGTA. The cytosol fraction was obtained by centrifugation of the homogenate at 100,000 x g for 30 min. In order to remove endogenous NADPH and T_3, the cytosol was extracted with charcoal as described(2). Protein concentration was measured by the method of Lowry et al.(9). DNA was measured by the method of Burton(10).

[^{125}I]T_3 Binding Assay: Charcoal-treated cytosol fraction, which was equivalent to 1.0 μg DNA, was incubated with 100,000 cpm [^{125}I]T_3(3,000 μCi/μg)(New England Nuclear Boston, MA) in the absence or presence of 50 uM NADPH(tetra sodium salt)(Sigma Chemical Co., St. Louis, MO) for 30 min at 0°C. This concentration of NADPH was enough to maximally activate the NADPH-dependent T_3 binding(2,3). In studies with Scatchard analysis, incubation for T_3 binding assay was performed in the presence of various concentrations(0-10^{-6}M) of unlabeled T_3(Sigma). After incubation, [^{125}I]T_3 bound to CTBP was determined by the method using dextran-coated charcoal as previously described(2,3).

RESULTS

In vivo Effect of Deprivation of Vitamin D on The NADPH-Dependent CTBP

By treatment of rats with vitamin D deficient diet, the NADPH-dependent T_3 binding activity was markedly reduced(Fig.1). The decrease in the binding activity was observed both in kidney and liver. During the

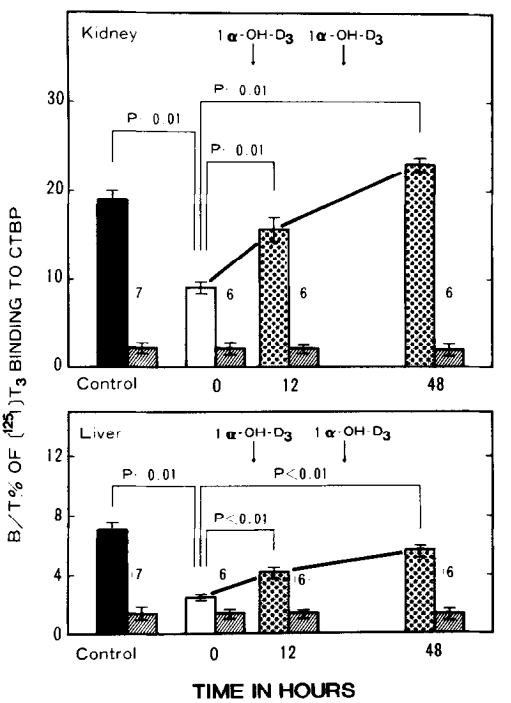

Fig. 1. Effect of 1α-OH-vitamin D_3 on the NADPH-dependent cytosolic T_3 binding activity in rat deprived from vitamin D.
Cytosol fractions were obtained at 0, 12, and 48 hrs after administration of 1α-OH-vitamin D_3 from rats treated with deprivation from vitamin D. Control cytosol was obtained from rats given regular diet. ▨ indicates the [^{125}I]T_3 binding in the absence of NADPH. Each value indicates the mean ± SD. The number in each column indicates the number of animals.

Table 1. Effect of administration of 1α-OH-D_3 on the serum concentration of calcium in vitamin D-deprived rats

Groups	Vitamin D deprivation	Injection	Serum Calcium Concentration(mg/100 ml)	P values
A (7)	(-)	Ethanol (2 times)	9.8 ± 0.7	A vs. B; NS.
B (6)	(+)	Ethanol (2 times)	9.6 ± 0.8	
C (6)	(+)	1α-OH-D_3(1 time)	10.1 ± 0.7	B vs. C <0.05
D (6)	(+)	1α-OH-D_3(2 times)	10.2 ± 0.7	B vs. D <0.05

Animals were treated as described in Material and Methods. Each value indicates mean ± SD.

deprivation from vitamin D, body weight of the animals decreased slightly but significantly(from 100.0 ± 2.1 to 92.1 ± 2.2 %). However, the serum concentration of Ca^{2+} was not modified by the treatment(Table 1).

In vivo Effect of 1α-OH-Vitamin D_3 on The NADPH-Dependent CTBP

The decreased T_3 binding activity was restored by administration of 1α-OH-D_3. In kidney, the binding activity was recovered within 12 hrs after the injection of 1α-OH-D_3. The value was further increased by additional injection of 1α-OH-D_3 (Fig.1). In contrast, the recovery of the CTBP was slow in liver. The level of CTBP slightly but significantly increased at 12 hrs after the injection of 1α-OH-D_3. The binding activity was continuously increased by further administration of 1α-OH-D_3(Fig.1). During the administration of 1α-OH-D_3, the serum concentration of Ca^{2+} slightly increased(Table 1).

Effect of 1,25-$(OH)_2$-vitamin D_3 on The NADPH-Dependent CTBP in Cultured dRLh Cells

When the dRLh cells were incubated in the presence of 10^{-8}M 1,25-$(OH)_2$-D_3, the T_3 binding activity significantly increased(Fig.2). Scatchard analysis showed that the maximal binding capacity(MBC) but not the affinity constant(Ka) for T_3 binding was increased by the treatment of the cells with 1,25-$(OH)_2$-D_3. The significant increase was observed even by 10^{-10}M 1,25-$(OH)_2$-D_3. The level of T_3 binding activity linearly increased in a time-dependent fashion, and was significantly higher at 6 hrs

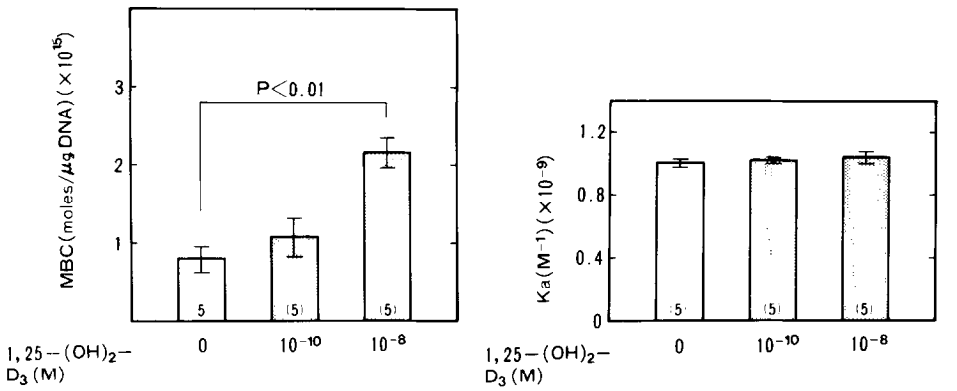

Fig. 2. Effect of 1,25-$(OH)_2$-vitamin D_3 on the level of NADPH-dependent cytosolic T_3 binding activity in cultured dRLh cells. Cytosol fraction was obtained from dRLh cells incubated in the absence or presence of 10^{-10} or 10^{-8}M 1,25-$(OH)_2$-vitamin D_3 for 12 hrs. Data obtained from the competition-inhibition were replotted by the method of Scatchard(11), and the maximal binding capacity(MBC)(Bo) and affinity constant(Ka) were estimated. Each value indicates the mean ± SD of 4 determinations.

incubation with 10^{-9}M 1,25-$(OH)_2D_3$(Fig.3). The 1,25-$(OH)_2$-D_3-induced increase in the NADPH-dependent T_3 binding was inhibited by addition of 0.1 μg/ml actinomycin D to the incubation medium(Table 2).

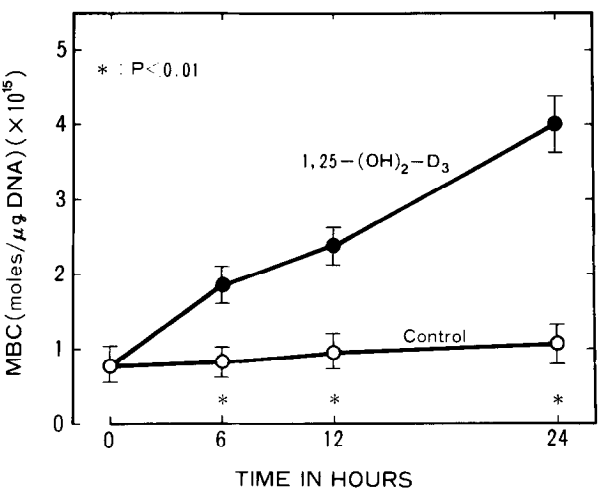

Fig. 3. Changes in the maximal binding capacity of NADPH-dependent cytosolic T_3 binding in dRLh cells during incubation with 1,25-$(OH)_2$-D_3. Cells were incubated in the absence(control) or presence of 10^{-9}M 1,25-$(OH)_2$-vitamin D_3 for indicated times. The maximal binding capacity(MBC) in each cytosol fraction was estimated by Scatchard analysis as described. Each value indicates the mean ± SEM of four determinations. *: P<0.01 between the cells treated with 1,25-$(OH)_2$-D_3 and control cells

Table 2. Effect of actinomycin D on the [^{125}I]T_3 binding activity in dRLh cells

Groups	No.	Actinomycin D (0.1 μg/ml)	1,25-$(OH)_2$-D_3 (10^{-9}M)	[^{125}I]T_3 binding (cpm/10^6 cells)	
				NADPH(25 μM)(-)	NADPH(25 μM)(+)
A	2	(-)	(-)	372	1758
B	2	(-)	(+)	408	3486
C	2	(+)	(-)	384	532
D	2	(+)	(+)	412	765

dRLh cells were incubated with or without actinomycin D for 2 hrs, and further incubated in the absence or presence of 1,25-$(OH)_2$-D_3 for 12 hrs. Each value indicates the mean of duplicate determinations of the specific binding of [^{125}I]T_3, in which the non-specific binding was defined as the radioactive T_3 bound in the presence of 10^{-6}M unlabeled T_3.

DISCUSSION

In this study, we have demonstrated that the binding capacity for T_3 in NADPH-dependent CTBP was increased by active vitamin D_3. During administration of 1α-OH-D_3 serum calcium concentration also increased in rats. Therefore, whether the increase of maximal binding capacity in the CTBP was caused from the increase in calcium concentration or from direct action of active vitamin D_3 was not certain. We observed that actinomycin D inhibited the 1,25-$(OH)_2$-D_3-induced increase in maximal binding capacity in cultured dRLh cells, suggesting that the increase was due to the stimulation of protein synthesis but was not dependent on calcium.

As we previously reported, the NADPH-dependent CTBP plays a role to actively transport T_3 to nuclear receptors from cytoplasm when the protein was activated by NADP(4,5). Thus, T_3 translocation may be regulated by vitamin D_3 through the increase in the amount of NADPH-dependent CTBP. However, the precise mechanism of the active vitamin D_3 regulation of CTBP-dependent T_3 translocation remains to be elucidated.

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