ROLE OF VITAMIN B_6 IN REGULATION OF INTERACTION OF $1,25(OH)_2D_3$ (CALCITRIOL) RECEPTORS WITH CHROMATIN AND DNA

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UDC 615.356:577.164.13-015.4.44.07

KEY WORDS: vitamin B_6, calcitriol and its receptors, chromatin, DNA

Calcitriol, or $1,25(OH)_2D_3$ (the hormonal form of vitamin D) has the functions, in particular, of maintaining extra- and intracellular calcium homeostasis, cell proliferation and differentiation, the immune response, and hormone production through interaction with a specific nucleophilic receptor [1, 8, 15]. The structure of this receptor is homologous with that of other steroid and thyroid hormones and vitamin A [9].

Regulation of binding with the $1,25(OH)_2D_3$ receptor and also of hormone-receptor complexes with chromatin has not been adequately studied. An increase in the concentration of $1,25(OH)_2D_3$ receptors is observed under the influence of the hormone itself [7, 12], of glucocorticoids [5], and retinoids [13], whereas a decrease in their affinity is observed in the presence of phosphorus deficiency [4]. To activate interaction of complexes of $1,25(OH)_2D_3$-receptors with chromatin, they must be modified by phosphorylation [14]. Vitamin K mediates Ca-dependent regulation of binding of $1,25(OH)_2D_3$ receptors with DNA [3].

Pyridoxal-5'-phosphate (PALP), the coenzyme form of vitamin B_6 interacts with receptors of steroid hormones in vitro, inhibiting their binding with DNA [6]. It has been shown [10] that PALP, like buffers with high ionic strength, solubilizes $1,25(OH)_2D_3$ receptors.

We accordingly decided to study the role of vitamin B_6 in the regulation of $1,25(OH)_2D_3$ reception in vivo. For this purpose we determined the concentration of $1,25(OH)_2D_3$ receptors (vacant and occupied in vivo) and binding of hormone-receptor complexes with DNA-cellulose in rats receiving different amounts of vitamin B_6. We also evaluated effects of PALP in vitro on binding of $1,25(OH)_2D_3$ receptors with DNA in the presence of vitamin B_6 deficiency. This paper describes data indicating a role of vitamin B_6 in the regulation of binding of $1,25(OH)_2D_3$ receptors with chromatin and DNA.

EXPERIMENTAL METHOD

Male Wistar rats initially weighing about 50 g were used. For 1 month the animals were kept on diets providing and deficient in vitamin B_6 and containing 0.60% of Ca and 1.23% of P [2]. The content of vitamin B_6 (pyridoxine chloride) in the diet was 10 mg/kg, and vitamin D_3 was given perorally in a dose of 1 μg (40 IU) per rat on alternate days.

Concentrations of vacant and endogeneously (in vivo) occupied $1,25(OH)_2D_3$ receptors in the chromatin fraction and cytosol of the small intestinal mucosa were determined by methods described previously [2, 3, 11] with modifications. The $1,25(OH)_2D_3$ receptors were extracted from the chromatin fraction or homogenate of the mucosa with hypertonic TED-KCl buffer (10 mM Tris-HCl; 1.5 mM EDTA; 1.0 mM dithiothreitol — DTT, 0.3 phenylmethylsulfonyl fluoride, pH 7.4). To determine the concentration of vacant nuclear $1,25(OH)_2D_3$ receptors aliquots of the high-speed supernant of the chromatin suspension (100 μl, 0.1 mg protein) in TED-KCl buffer were incubated for 16 h at 0-4° with ^3H-$1,25(OH)_2D_3$ in a saturating concentration (1.0 nM) of a ±250-fold excess of unlabeled $1,25(OH)_2D_3$. The total concentration of receptors of the hormone was measured after treatment of the receptor preparations with L-tosylamide-2-phenylchloromethylketone (200 μM) followed by incubation at 37°C with ^3H-$1,25(OH)_2D_3$ in a saturating concentration for 30 min. The level of occupied $1,25(OH)_2D_3$ receptors in vivo was calculated as the difference between the concentrations of total and vacant receptors. The concentration of total $1,25(OH)_2D_3$ receptors in the cytosol also was measured by a volumetric method, but at 0-4°C and after blockade of the

Institute of Nutrition, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 12, pp. 631-632, December, 1990. Original article submitted December 18, 1989.

0007-4888/90/0012-1689$12.50

TABLE 1. Concentration of Nuclear $1,25(OH)_2D_3$ Receptors in Small Intestinal Mucosa of Rats with Different Vitamin B_6 Intakes ($M \pm m$)

$1,25(OH)_2D_3$ receptors	Group of animals	
	$+B_6$	$-B_6$
Vacant, fmoles/mg	43,4±4,6	52,0±3,8
Occupied, fmoles/mg	15,0±1,9	26,2±2,7*
Occupied, %	25,7±2,2	33,5±1,7*

Legend. Here and in Table 2: $+B_6$ and $-B_6$) animals receiving and deprived of vitamin B_6; *$p < 0.05$ between groups.

TABLE 2. Concentration of Cytoplasmic $1,25(OH)_2D_3$ Receptors in Small Intestinal Mucosa and Binding of $1,25(OH)_2D_3$-Receptor Complexes with DNA-Cellulose in Rats with Different Vitamin B_6 Intakes ($M \pm m$)

Parameter	Group of animals	
	$+B_6$	$-B_6$
$1,25(OH)_2D_3$ receptors		
Vacant, fmoles/mg	279±6 (223±8**)	258±20 (212±12)
Occupied, fmoles/mg	73±4 (79±3)	96±3* (104±5)
Occupied, %	20,7±1,3	27,1±2,2*
Binding with DNA-cellulose, %	47,0±4,7 (12,2±1,6**)	66,7±4,0* (19,8±2,3**)

Legend. Values of parameters determined after addition of PALP, and significance of their difference (**$p < 0.05$) from the corresponding parameter, measured without addition of PALP, given in parentheses.

SH-groups of the receptor by a mercury-containing reagent, followed by their reduction with DTT [11]. Aliquots of high-speed (105,000*g*, 1 h, 4°C) cytosol (200 μl, 0.25 mg protein) were incubated for 16 h at 0-4°C with ^3H-$1,25(OH)_2D_3$ + $1,25(OH)_2D_3$. The labeled cytosol (100 μl) was then incubated for 1 h at 0-4°C with mersalyl acid (1.0 mM). The receptor preparations were then treated with suspensions of charcoal (0.5%) coated with dextran (0.05%), to adsorb the displaced ligand. Next an excess of DTT (2.0 mM), ^3H-$1,25(OH)_2D_3$ ± $1,25(OH)_2D_3$ was added to the supernatant, and the sample was incubated for 4 h at 0-4°C. Samples treated with mersalyl acid were used to determine the total concentration of receptors not containing the displaced reagent — to determine the concentration of vacant receptors. During determination of the kinetics of binding of $1,25(OH)_2D_3$ with nuclear and cytoplasmic receptors the concentration of labeled $1,25(OH)_2D_3$ was 0.0625-1.0 nM. Separation of the free and bound ligands was carried out in all cases by a method using hydroxyapatite [2].

To assess binding of $1,25(OH)_2D_3$-receptor complexes with heterologous DNA [3] we used cytoplasmic receptors, labeled for 16 h, to which a suspension of DNA-cellulose (50% *w/v*) in TED buffer was added up to a KCl concentration of 0.1 M, after which the sample was incubated for 1 h at 0-4°C. PALP was added to the receptor preparations in a final concentration of 4 mM 15 min before addition of the isotope.

The serum concentration of $1,25(OH)_2D_3$ was determined by the radio-competitive receptor binding method using kits of the "1-25-Dihydroxyvitamin D (^3H) Assay Reagents System" (Amersham), after isolation of the hormone fraction by HPLC.

EXPERIMENTAL RESULTS

Vitamin B_6 deficiency in rats led to a marked increase (by 1.75 times) in the concentration of nuclear $1,25(OH)_2D_3$ receptors occupied in vivo in the small intestinal mucosa (Table 1). The same pattern was observed when the concentration of

occupied receptors was measured in the cytosol of the mucosa (an increase of 31.5%, Table 2), whereas the relative percentage of occupied receptors in both cases increased by 1.3 times.

The concentration of vacant nuclear and cytoplasmic $1,25(OH)_2D_3$ receptors in vitamin B_6-deficient animals showed no significant change (Tables 1 and 2) and correspondingly there was no change in their maximal capacity (B_{max} of the nuclear receptors was 55-65 fmoles/mg, and of the cytoplasmic receptors 310-340 fmoles/mg). The affinity of the receptors for the hormone likewise did not vary significantly (the equilibrium dissociation constant of the nuclear receptors was K_d = 0.09-0.10 nM, and of the cytoplasmic K_d = 0.12-0.14 nM).

Binding of $1,25(OH)_2D_3$ receptors occupied in vivo with DNA-cellulose increased in animals with vitamin B_6 deficiency by 42% (Table 2). PALP, added in vitro to the extracted cytoplasmic $1,25(OH)_2D_3$ receptors reduced binding of the ligand by vacant receptors (by 15-25%) and depressed association of hormone-receptor complexes with DNA-cellulose (by 3.5-4.5 times) in rats both receiving and deficient in vitamin B_6. The concentration of cytoplasmic $1,25(OH)_2D_3$ receptors occupied in vivo was not reduced after addition of PALP, i.e., PALP did not cause dissociation of hormone-receptor complexes formed in vivo.

The concentration of $1,25(OH)_2D_3$ in the serum of rats with vitamin B_6 deficiency not only was not increased, but it actually had a tendency to decrease (103 ± 9 compared with 160 ± 36 pg/ml). This indicates that an increase in the proportion of occupied $1,25(OH)_2D_3$ receptors cannot be explained as the result of an increase in the circulating hormone concentration.

These data clearly demonstrate increased binding in vivo of complexes of the $1,25(OH)_2D_3$-receptor with chromatin and DNA in vitamin B_6 deficiency, and also some degree of inhibition of the formation of these complexes by the action of PALP in vitro.

The mechanism of regulation of binding of $1,25(OH)_2D_3$ receptors with chromatin and DNA by vitamin B_6 may lie in a covalent modification by PALP of the receptor protein as a result of Schiff base formation. A lysine enriched sequence can be distinguished in the DNA-binding domain of the receptor [9], where interaction between PALP and the receptor protein probably takes place. This, in turn, may impede interaction of the hormone-receptor complexes with chromatin (and partly of $1,25(OH)_2D_3$ with the receptor) and/or it may be the signal for termination of this interaction (for example, by inducing solubilization of the receptors).

Our results thus demonstrate for the first time that vitamin B_6 is a physiological regulator of binding of $1,25(OH)_2D_3$ receptors with chromatin and DNA, depressing the increase in the concentration of receptors occupied in vivo and, consequently, blocking potentiation of the hormonal response.

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