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RECEPTOR FOR 1,25-DIHYDROXYVITAMIN D IN A VASCULAR SMOOTH MUSCLE CELL LINE DERIVED FROM RAT AORTA

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The presence of a specific receptor for 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) was investigated in a cell line A7r5 derived from fetal aorta. $1,25(OH)_2[^3H]D_3$ binding to cytosol was saturated at 0.6-1 nM, and Scatchard analysis yielded dissociation constant and binding sites, $(3.02 \pm 0.4) \times 10^{-11}$ M and 33.9 $\pm$ 5.8 fmol/mg protein, respectively. Sucrose density gradient analysis revealed the sedimentation constant 3.2S. Furthermore, the receptor protein had affinity for DNA-cellulose column and eluted with 0.2 M KCl. These data suggest that vascular smooth muscle cell may be a target tissue of vitamin D.

Essential role of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) is known to regulate extracellular calcium homeostasis. In addition to this well-known classical action of vitamin D, $1,25(OH)_2D$ has been demonstrated to induce cell differentiation and modulation of immune system (1, 2). Recently, skeletal (3, 4) and cardiac (5) myocytes have been added to possible target organs for $1,25(OH)_2D$ since these cells are found to have receptor for the hormone. In the skeletal muscle cells, it is known that $1,25(OH)_2D$ stimulates cellular calcium uptake and Ca-ATPase activity (6). No evidence has been reported whether or not vascular smooth muscle cells have receptor for the hormone. Recently attentions have been focused on the disturbances in the intracellular as well as extracellular calcium

Abbreviations used: $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; $24,25(OH)_2D_3$, 24,25-dihydroxyvitamin D_3; $25(OH)D_3$, 25-hydroxyvitamin D_3; DMEM, Dulbecco's modified Eagle's medium. SH, spontaneously hypertensive; WKy, Wistar Kyoto; IMCAL, integrated membrane calcium-binding protein; $1,25(OH)_2D$ refers to a mixture of the corresponding metabolite of cholecalciferol and ergocalciferol.

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metabolism in hypertensive animals including humans (7-9). An increase in renal production as well as breakdown of $1,25(OH)_2D$ has been reported in the spontaneously hypertensive (SH) rats even before the onset of hypertension and thus $1,25(OH)_2D$ level in plasma is maintained in the normal range (10). It has also been reported by Kowarski and his associates that vitamin D-dependent integrated membrane calcium binding protein (IMCAL) content in the various tissues is reduced by 30% in SH rats as compared with that in normotensive Wistar Kyoto (WKy) rats (11) and that this is in parallel with the decreased membrane calcium binding of the corresponding cells (7, 12). These results, taken together with the idea that elevated vascular tone due to increased intracellular ionized calcium concentration is a primary cause of hypertension, suggested me that $1,25(OH)_2D$-mediated process may be involved in the disturbances in cellular calcium handling in hypertension. If this is the case, one might predict presence of $1,25(OH)_2D$ receptor in vascular smooth muscle cells, since the sterol hormone is known to act through its receptor activating a genomic process.

The present study was, therefore, aimed at examining presence of receptor for $1,25(OH)_2D$ in vascular smooth muscle cells. I report here that a vascular smooth muscle cell line, A7r5 (CRL-1444), derived from fetal rat aorta, has receptors for the sterol and may have a role in the maintenance of intracellular calcium homeostasis.

MATERIALS AND METHODS

Tissue culture: A cell line, A7r5, was purchased from the American Type Culture Collection, and incubated in DMEM containing 10% fetal calf sera at 37°C in the atmosphere of 95% O_2 and 5% CO_2. At subconfluence, cells were detached by the treatment with trypsin EDTA (Gibco) for 3 minutes. Cells were collected, washed three times with Hanks' solution, and homogenized in the TKED buffer (50 mM Tris-HCl, 300 mM KCl, 1.5 mM EDTA, and 5.0 mM dithiothreitol, pH 7.4) by a three burst of sonicator. The homogenates were centrifuged for 60 min at 105,000 x g and 4°C to prepare cytosol. The cytosol was stored at -70°C until use.

Equilibrium binding studies: Aliquots of cytosol (0.5 mg protein/0.5 ml) was incubated for 18 hr at 4°C with the graded amount of $1,25(OH)_2$[26,27-methyl-^{3}H]D_3 (specific activity: 160 Ci/mmol, Amersham, Arlington Heights,

IL, U.S.A.) in the presence or absence of excess amount (200-fold) 1,25(OH)$_2$D$_3$ (generously given by Dr. Uskokovic of Hoffman-La Roche, Nuttley, NJ, U.S.A.). Each tube received 200 µl of dextran-coated charcoal, incubated for additional 10 min, and centrifuged for 10 min at 4°C and 2000 x g. The supernatant was transferred to a vial and radioactivity was determined by means of liquid scintillation counter. Specific binding was obtained by subtracting non-specific binding from the total binding. Binding data were obtained by the Scatchard analysis.

Sucrose density gradient analysis: Aliquots of receptor (1 mg protein/ml) were incubated at 4°C for 3 hr with 1,25(OH)$_2$[^{3}H]D$_3$ in the presence or absence of excess (100-fold) amount of 1,25(OH)$_2$D$_3$. Alternatively, a 100-fold excess of unlabeled 24,25-dihydroxyvitamin D$_3$ (24,25(OH)$_2$D$_3$) (a gift from Dr. Uskokovic of Hoffman-La Roche) or 25-hydroxyvitamin D$_3$ (25(OH)D$_3$) (kindly provided by the Upjohn Co., Kalamazoo, MI, U.S.A.) were incubated with 1,25(OH)$_2$[^{3}H]D$_3$ to test specificity of the binding. Bound hormone was separated from free by absorption of free hormone to dextran-coated charcoal as described above and 0.2 ml aliquots were put on the top layer of the discontinuous sucrose gradient (4-20%sucrose in KTED buffer) and centrifuged for 20 hr at 260,000 x g and 4°C. Fractions (0.2 ml each) were collected from the bottom and counted for the radioactivity. As a molecular size marker, ^{14}C-ovalbumin was added to each tube.

DNA cellulose chromatography: Cytosol was prepared in TKED buffer. DNA-cellulose was purchased from Sigma Chemicals. Cytosol was labeled with 1 nM 1,25(OH)$_2$[^{3}H]D$_3$ at 4°C for 3 hr, and bound hormone was separated from free as described above. The labeled cytosol was then diluted 6-fold in TED buffer (50 mM Tris-HCl, 1.5 mM EDTA, 5.0 mM dithiothreitol, pH 7.4). One milliliter of cytosol was incubated for 30 min at 0°C with a 2-ml packed volume of DNA-cellulose in a batch technique. Then the DNA-cellulose slurry was packed into 3-ml plastic syringe, and the column was rinsed with 20 ml of TED buffer and eluted with a 40-ml 0.05-0.5 M KCl gradient.

RESULTS

As depicted in the Figure 1a, binding of 1,25(OH)$_2$[^{3}H]D$_3$ to cytosol was saturated in the range of 0.5-1.0 nM. Scatchard analysis revealed the presence of a single class of binding site (Figure 1b). The dissociation constant (K_d) and binding sites were (3.02 $\pm$ 0.4) x 10^{-11} M and 33.9 $\pm$ 5.8 fmol/mg protein, respectively.

Figure 2 illustrates sucrose density gradient analysis of 1,25(OH)$_2$D$_3$-receptor complex. The binding was specific for 1,25(OH)$_2$D$_3$ and sedimentation constant was approximately 3.2S.

The chromatographic profile of receptor for 1,25(OH)$_2$D on DNA cellulose is shown in Figure 3. Approximately 50% of 1,25(OH)$_2$[^{3}H]D$_3$ binding proteins is shown to possess affinity for DNA-cellulose and is eluted from this column by 0.2 M KCl. A radioactive peak, approximately 50% of total, was noted in the void volume of this column.

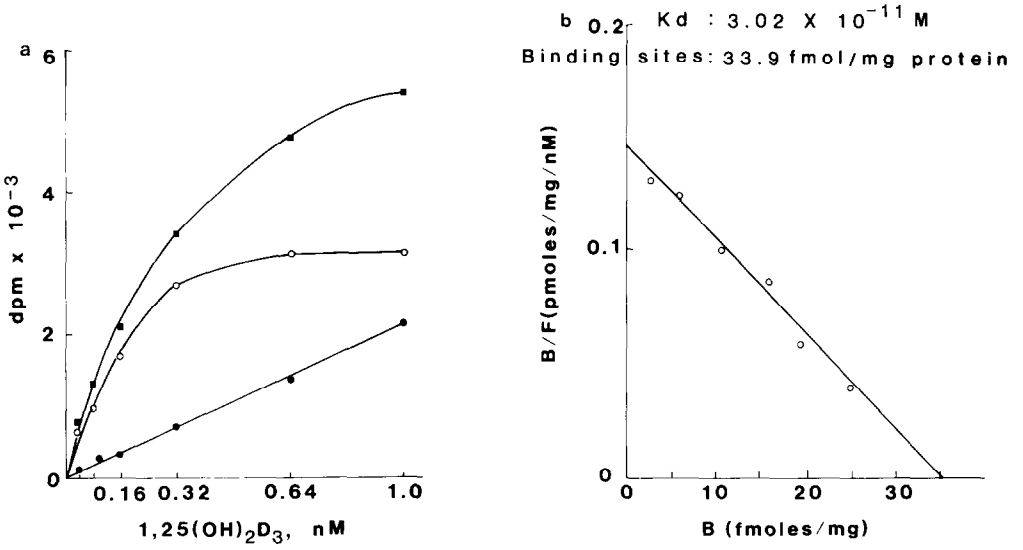

Figure 1. Saturation analysis and Scatchard analysis of specific 1,25(OH)2[^{3}H]D3 binding in cytosol from cultured vascular smooth muscle cells (A7r5). (a)Saturation analysis. Total binding (□—□), specific binding (○—○) and non-specific binding (●—●). (b)Scatchard analysis. For details, see the text.

DISCUSSION

The present study clearly demonstrates the presence of 1,25(OH)2D receptor in the vascular smooth muscle cells. The characteristics of the

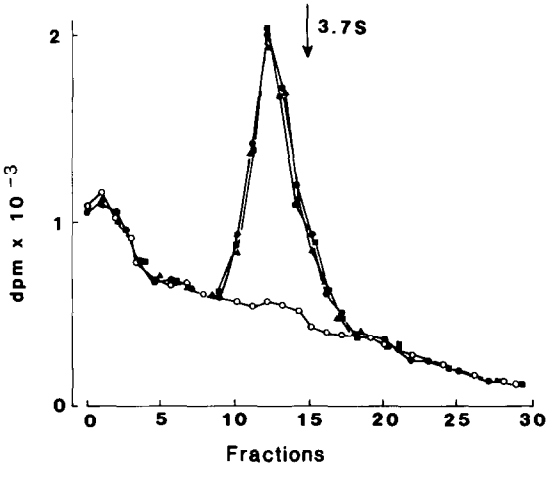

Figure 2. Sucrose density gradient analysis (0.12-ml fractions) of 1,25(OH)2[^{3}H]D3 binding to vascular smooth muscle cell (A7r5) cytosol preparations. Aliquots of cytosol protein (1mg) as described under "MATERIALS AND METHODS" were incubated with 1 nM 1,25(OH)2[^{3}H]D3 alone (●) or in the presence of 100-fold molar excess of non-radioactive 1,25(OH)2[^{3}H]D3 (○), 24,25(OH)2D3 (△), or 25(OH)D3 (□). A part of this cytosol (200 µl) was analyzed on 4-20% sucrose gradients in TKED buffer.

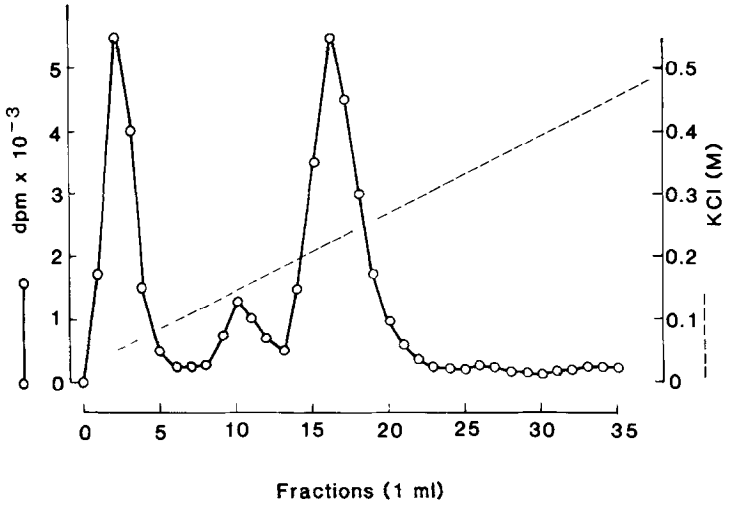

Figure 3. DNA-cellulose chromatography of the 1,25(OH)$_2$D receptor from A7r5 vascular smooth muscle cell cytosol. A7r5 cytosol (3 mg protein/ml)was incubated with 1 nM 1,25(OH)$_2$D$_3$ at 4°C for 3 hr and bound 1,25(OH)$_2$D$_3$ was separated from free using dextran-coated charcoal. A 1-ml sample was diluted to 6 ml in TED buffer and applied to a DNA-cellulose column (2ml) at a flow rate of 30 ml/hr. The receptor was eluted with a 40-ml 0.05-0.5 M KCl gradient.

1,25(OH)$_2$D receptor in this study are similar to the intestinal receptor for 1,25(OH)$_2$D. This conclusion is suggested by metabolite specificity, sucrose density gradient sedimentation, ligand binding affinity, and DNA binding. The dissociation constant and number of binding sites per mg protein are also similar to those of recent studies in skeletal and cardiac muscle cells (3-5).

It has been suggested that 1,25(OH)$_2$D has a role in regulating cellular calcium homeostasis affecting such as Ca-ATPase and calcium uptake by sarcoplasmic reticulum (4-6). It is also suggested that 1,25(OH)$_2$D may affect muscle cell differentiation (4). Although no evidence is available suggesting that 1,25(OH)$_2$D may have a direct action on vascular smooth muscle cells, it is interesting to note the impaired vitamin D metabolism (10) as well as decreased IMCAL in various tissues (11) in the SH rats. The latter may be related to the reduction in calcium binding capacity of the membrane reported (8, 12) and may be one of factors which lead to the elevated intracellular ionized calcium concentration (7-9). This, in turn, may lead to increase vascular tone thereby elevate blood pressure.

Preliminary data in our laboratory suggest that 1,25(OH)$_2$D indeed increase Ca-ATPase activity in the A7r5 cells which have receptors for 1,25(OH)$_2$D as shown in the present study. Thus further investigations of the action of 1,25(OH)$_2$D on vascular smooth muscle function may provide new information and insight into vascular smooth muscle function in health and diseases such as hypertension.

In summary, the present study demonstrates for the first time that vascular smooth muscle cell has receptor for 1,25(OH)$_2$D$_3$ and suggests a role in regulating cellular calcium metabolism.

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