**1,25-DIHYDROXYVITAMIN D RECEPTOR AND ITS BIOLOGICAL ROLE IN VASCULAR SMOOTH MUSCLE CELL FUNCTIONS**

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INTRODUCTION

It is well established that 1,25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$) has a major role in the regulation of calcium homeostasis. To achieve this, 1,25(OH)$_2$D$_3$ binds to its receptor followed by activating gene expression, which in turn, induce new protein synthesis such as calcium binding protein leading to cellular functions. The series of event have been established in the intestine, kidney and bone, which are principal tissues responsible for maintaining calcium homeostasis (1).

In addition to these well-known classical effects of 1,25(OH)$_2$D$_3$ in the regulation of the extra-cellular calcium metabolism, accumulating evidence suggests that 1,25(OH)$_2$D$_3$ has a variety of cellular functions in different types of tissues including cell differentiation, immunomodulation, and so on (2,3). Calcium is one of the most important factors not only in cellular functions but also in the signal transduction of many hormones and agents. Disturbances in both intra-cellular and extra-cellular calcium homeostasis has been known in the hypertension in animals as well as humans (4-6). However, the exact mechanism which lead to the hypertension is not known. The favorable effect of oral calcium supplement to hypertension has been seemingly controversial. However, since calcium, per se, can not be attributable to the effect of calcium supplement, other factors may possibly be responsible for the effect. These can be any factors, but calcium regulating hormones are most probable. We have demonstrated recently that vitamin D metabolism is impaired in the spontaneously hypertensive rat (SHR), the genetic model of the essential hypertension (7). We have also found that 1,25(OH)$_2$D$_3$ receptor in the kidney was reduced in the SHR, which may at least in part, explain the reported calcium leak in this animal model as well as in the essential hypertension (8). In addition, Kowarski et al., recently demonstrated that the integrated membrane calcium binding protein (IMCAL), which is vitamin D dependent, is reduced in various tissues in the SHR, and this is in parallel with the reduced calcium binding capacity of cell membrane in this animal model of hypertension (9). These data strongly suggest that vitamin D dependent process may be also involved in the cellular calcium handling, and disturbances in this function may lead to the development or exaggeration of hypertension, since intracellular calcium is the most
probable cause of increased vascular tone. If vitamin D is involved in the cellular calcium regulation, it is natural that vitamin D-related parameters may appear abnormal. Since a receptor mediated event has been always the way this hormone functions, it is reasonable to assume that any target tissue must have receptor for 1,25(OH)2D3. This led us to search for 1,25(OH)2D3 receptor in a vascular smooth muscle cell. Here I report in the present paper that a vascular smooth muscle cell line, A7r5, which is derived from fetal rat aorta, has receptors for 1,25(OH)2D3. I will also discuss possible functions of the receptor in the regulation of intracellular calcium in relation to hypertension.

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 serum at 37°C in the atmosphere of 95% O2 and 5% CO2. At subconfluence, cells were detached by the treatment with trypsin EDTA (Gibco) for 3 minutes. Cells were washed, washed three times with Hanks' solution, and homogenized in the KTED buffer (50 mM Tris-HCl, 300 mM KCl, 1.5 mM EDTA, and 5.0 mM dithiothreitol, pH 7.4) by three bursts 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 graded amount of 1,25(OH)2[26,27-methyl-3H]D3 (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)2D3 (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[3H]D3 in the presence or absence of excess (100-fold) amount of 1,25(OH)2D3. Alternatively, a 100-fold excess of unlabeled 24,25-dihydroxyvitamin D3 (24,25(OH)2D3)(a gift from Dr. Uskokovic of Hoffman-La Roche) or 25-hydroxyvitamin D3 (25-(OH)D3)(kindly provided by the Upjohn Co., Kalamazoo, MI, U.S.A.) were incubated with 1,25(OH)2[3H]D3 to test specificity of the binding. Bound hormones were separated from free by absorption of free hormones 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, C-ovalbumin was added to each tube.

DNA cellulose chromatography: Cytosol was prepared in KTED buffer. DNA-cellulose was purchased from Sigma Chemicals. Cytosol was labeled with 1 nM 1,25(OH)2[3H]D3 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.