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# Vitamin D: a negative endocrine regulator of the renin–angiotensin system and blood pressure☆

Yan Chun Li[a,*], Guilin Qiao[a], Milan Uskokovic[b], Wei Xiang[a], Wei Zheng[a], Juan Kong[a]

[a] *Department of Medicine, University of Chicago, MC 4076, 5841 S. Maryland Avenue, Chicago, IL 60637, USA*
[b] *BioXell Inc., Nutley, NJ 07110, USA*

**Abstract**

The renin–angiotensin system (RAS) plays a central role in the regulation of blood pressure, volume and electrolyte homeostasis. Inappropriate activation of the RAS may lead to hypertension. Clinical and epidemiological studies have suggested a correlation between Vitamin D-deficiency and high blood pressure. Our recent studies demonstrate that Vitamin D is a potent endocrine suppressor of renin biosynthesis to regulate the RAS. Mice lacking the Vitamin D receptor (VDR) have elevated production of renin and angiotensin (Ang) II, leading to hypertension, cardiac hypertrophy and increased water intake. These abnormalities can be prevented by treatment with an ACE inhibitor or $AT_1$ receptor antagonist. Vitamin D repression of renin expression is independent of calcium metabolism, the volume- and salt-sensing mechanisms and the Ang II feedback regulation. In normal mice, Vitamin D-deficiency stimulates renin expression, whereas injection of 1,25-dihydroxyvitamin $D_3$ [1,25$(OH)_2D_3$] reduces renin synthesis. In cell cultures, 1,25$(OH)_2D_3$ directly suppresses renin gene transcription by a VDR-dependent mechanism. Furthermore, we have found that Gemini compounds have more potent renin-suppressing activity than 1,25$(OH)_2D_3$. Collectively, our studies reveal a critical role of the Vitamin D endocrine system in the regulation of blood pressure and volume homeostasis, and suggest that low calcemic Vitamin D analogs may potentially be developed into a new class of anti-hypertensive agents to control renin production and blood pressure.


*Keywords:* Vitamin D; Vitamin D analogs; Renin; Angiotensin; Hypertension

## 1. Introduction

It is long established that the primary physiological role of the Vitamin D endocrine system is to regulate calcium homeostasis by regulating intestinal and renal calcium transport and bone mineralization [1]. As the most active metabolite of Vitamin D, 1,25-dihydroxyvitamin $D_3$ [1,25$(OH)_2D_3$] is responsible for most of the biological actions of the Vitamin D endocrine system, which are mediated by the Vitamin D receptor (VDR), a member of the nuclear receptor superfamily [2]. Since VDR is also widely expressed in tissues not involved in calcium metabolism, it is believed that the Vitamin D endocrine system has additional physiological functions beyond calcium homeostasis. This notion is confirmed by recent studies from many laboratories. For example, studies using genetically mutant mice that lack VDR or 25-hydroxyvitamin $D_3$ 1α-hydroxylase, the rate-limiting enzyme for 1,25$(OH)_2D_3$ biosynthesis, show that Vitamin D/VDR regulate not only calcium homeostasis as expected, but also immune responses, reproduction, mammary gland development and hair growth [3–7]. Our recent works demonstrate that 1,25$(OH)_2D_3$ also functions as a negative endocrine regulator of the renin–angiotensin system (RAS) and thus plays an important role in the regulation of the renocardiovascular functions [8,9].

## 2. The renin–angiotensin system

The RAS is a regulatory cascade that plays an essential role in the regulation of blood pressure, electrolyte and volume homeostasis. Inappropriate stimulation of the RAS has been associated with hypertension. Renin, an aspartyl protease, is the rate-limiting component of the RAS. Its main function is to cleave a 10 amino acid peptide, angiotensin (Ang) I, from angiotensinogen. Ang I is then converted to the octapeptide Ang II by the angiotensin-converting enzyme (ACE), which primarily resides in endothelial cells in blood vessels. Ang II is the central effector of the RAS. Through interacting with Ang II receptors [10] in different tissues, including the brain, heart, kidney, adrenal glands

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* Corresponding author. Tel.: +1-773-702-2477; fax: +1-773-702-5790.
*E-mail address:* cyan@medicine.bsd.uchicago.edu (Y.C. Li).

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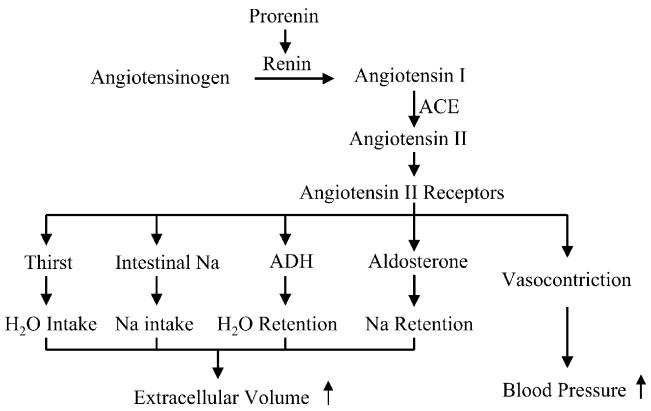


Fig. 1. The renin–angiotensin system. ADH, anti-diuretic hormone, also known as vasopressin; ACE, angiotensin-converting enzyme.

and peripheral vasculature, Ang II exerts diverse physiological responses that influence the electrolyte and extracellular volume balance and blood pressure (Fig. 1) [11]. In addition, local RAS has been found in tissues such as the kidney, heart and brain, among others [12], the physiological relevance of which remains to be determined. Adding a new level of complexity to the RAS, recently a renin receptor has been reported in the heart, kidney, brain, placenta and liver, which may function to increase the catalytic activity of renin [13], and an ACE homologue, ACE2, has been isolated [14,15] and found to play an essential role in heart functions [16].

Renin is predominantly produced and secreted by special granulated smooth muscle cells located in the afferent glomerular arterioles in the juxtaglomerular (JG) apparatus of the kidney [17]. Secretion of renin from the granulated smooth muscle cells, term JG cells, is controlled by physiological stimuli such as changes in renal vascular perfusion pressure, tubular sodium chloride load, and renal sympathetic nerve activity [11,17]. At the local level, renin secretion is influenced by a large number of bioactive factors such as prostaglandins, nitric oxide, adrenomedullin, Ang II (feedback), endothelin, vasopressin and adenosine [11,17,18]. At the molecular level, recent studies demonstrate that renin biosynthesis is regulated by an array of transcriptional factors [19–23]. These proteins influence renin gene transcription by a positive or negative fashion.

## 3. Clinical and epidemiological evidence linking Vitamin D-deficiency with hypertension

Accumulating evidence from epidemiological and clinical studies in the last decades has suggested a connection between Vitamin D and blood pressure. As ultraviolet (UV) irradiation is essential for the cutaneous production of Vitamin D, circulating Vitamin D levels are greatly influenced by geographic locations, seasonal changes and skin pigmentations. Data obtained from the INTERSALT study centers reveal a linear correlation between the rise in blood pressure or the prevalence of hypertension and the latitudes north or south of the equator [24]. Data from a national survey in China also demonstrate that the prevalence of hypertension and stroke incidence display a high-to-low gradient from the north to the south of the country [25]. Seasonal variations in blood pressure have been reported in temperate climates, with blood pressure higher in the winter (low UV irradiation) than in the summer (high UV irradiation) [26,27]. Dark skin pigmentation, which affects an efficient UV light penetration [28], has also been reported to be associated with higher blood pressure [29,30]. Indeed, UV irradiation has been reported to lower blood pressure in patients with mild essential hypertension [31].

Numerous studies have shown that the serum level of $1,25(OH)_2D_3$ is inversely associated with blood pressure in normotensive and hypertensive subjects [32–34]. More interestingly, such inverse relationship has also been reported between circulating $1,25(OH)_2D_3$ levels and plasma renin activity in patients with essential hypertension [35]. Vitamin D supplement has been reported to be beneficial to the heart. In clinical trials, Vitamin D treatment has been reported to reduce blood pressure in hypertensive or elderly patients [36,37]. In several cases, $1,25(OH)_2D_3$ treatment has been shown to reduce the blood pressure, plasma renin activity, Ang II level and myocardial hypertrophy [38,39].

## 4. Vitamin D as a negative endocrine regulator of the renin–angiotensin system

The clinical and epidemiological evidence suggests that Vitamin D may regulate blood pressure via regulating the RAS. Based on the inverse relationship between serum $1,25(OH)_2D_3$ levels and plasma renin activity found previously, we speculated that $1,25(OH)_2D_3$ might be a negative endocrine regulator of renin production in vivo. If this hypothesis is correct, disruption of the Vitamin D signaling pathway should lead to a deregulated stimulation of renin synthesis, whereas an increase in serum $1,25(OH)_2D_3$ levels should lead to renin suppression.

To test this hypothesis, we examined VDR-null mutant mice, reasoning that renin expression should be increased in these mice due to the lack of VDR-mediated Vitamin D signaling. Indeed, we found that both renin mRNA and protein levels in the kidney, as well as the plasma Ang II production, were drastically increased in VDR (−/−) mice, whereas the expression of angiotensinogen in the liver was the same as in wildtype mice. Thus, the increase in plasma Ang II appeared to be mainly due to an increase in renin activity. As a consequence of the aberrant RAS over-stimulation, VDR (−/−) mice developed hypertension, cardiac hypertrophy and an over-drinking behavior. Plasma and urinary aldosterone levels were also increased in VDR (−/−) mice. Cardiac hypertrophy, likely induced by Ang II, was reflected by a higher heart to body weight ratio and increased cardiac myocyte size in the ventricle revealed by histological analyses. Accompanying the cardiac hypertrophy, both cardiac

mRNA expression and plasma concentration of atrial natriuretic peptide (ANP) were found to be increased in VDR (−/−) mice, presumably as a compensatory mechanism.

The high blood pressure, cardiac hypertrophy and increased water intake seen in VDR (−/−) mice could be corrected by treatment with captopril, an ACE inhibitor, or losartan, an Ang II $AT_1$ receptor antagonist, confirming that over-stimulation of the RAS is indeed responsible for the abnormalities. Interestingly, captopril or losartan treatment resulted in a drastic up-regulation of renin expression in both wild-type and VDR (−/−) mice. We also showed that renin expression in both wild-type and VDR (−/−) mice was stimulated by dehydration, and suppressed by a high sodium diet. In all cases VDR (−/−) mice still maintained a significantly higher renin expression than wild-type mice. Therefore, despite a high basal renin synthesis, the regulatory mechanisms that control renin production, including the Ang II feedback inhibition and the volume- and salt-sensing mechanisms, are still intact in VDR (−/−) mice. Thus, the sustained renin up-regulation is mediated by other mechanisms.

VDR (−/−) mice develop hypocalcemia and secondary hyperparathyroidism [5], which may influence renin production and secretion. To address the contribution of serum calcium or parathyroid hormone (PTH) to renin up-regulation in VDR (−/−) mice, we found that renin up-regulation was already evident in 20-day-old VDR (−/−) mice, before the hypocalcemia developed, and persistent in normocalcemic adult VDR (−/−) mice after their blood calcium was normalized through dietary intervention. The plasma Ang II level remained elevated in the normocalcemic state. On the other hand, renin expression was normal in Gcm2 (−/−) mice [40], even though these mutant mice were as hypocalcemic as VDR (−/−) mice. In addition, renin expression was still elevated in VDR (−/−) mice whose alopecia was rescued by targeted expression of human VDR in the skin [41]. These date strongly suggest that regulation of renin expression by $1,25(OH)_2D_3$ is independent of calcium metabolism or alopecia. However, the contribution of PTH to the renin up-regulation in VDR (−/−) is less certain yet, because serum PTH starts to rise early in life before hypocalcemia develops and can not be completely normalized by dietary treatment, due to the lack of the VDR-mediated Vitamin D inhibition of PTH biosynthesis [42].

We also tested the hypothesis using wild-type mice. We showed that in wild-type mice rendered Vitamin D-deficient by dietary strontium treatment, which inhibits $1,25(OH)_2D_3$ biosynthesis [43], renin expression in the kidney was up-regulated as in VDR (−/−) mice. On the other hand, in wild-type mice receiving several doses of $1,25(OH)_2D_3$ injection renin expression was suppressed. Thus, the inhibitory role of $1,25(OH)_2D_3$ in renin biosynthesis was confirmed in normal mice.

We further tested the hypothesis using As4.1 cells, a JG cell-like cell line that was derived from kidney tumors in SV40 T antigen transgenic mice and maintains a high level of renin synthesis [44]. We found that $1,25(OH)_2D_3$ drastically reduced renin mRNA expression in As4.1 cells transiently or stably transfected with human VDR cDNA. To elucidate the molecular mechanism whereby $1,25(OH)_2D_3$ suppresses renin gene expression, the stably transfected cells were used to analyze the renin gene promoter by luciferase reporter assays. When the cells were transfected with a luciferase reporter plasmid containing a 4.1 kb 5′-flanking sequence of the murine Ren-1c gene, $1,25(OH)_2D_3$ treatment markedly reduced the promoter activity, confirming that $1,25(OH)_2D_3$ directly and negatively regulates renin gene transcription by a VDR-mediated mechanism. Deletion analysis of the Ren-1c gene promoter has identified two short fragments, from −2720 to −2642 and −117 to +1, that are required to mediate the repression by $1,25(OH)_2D_3$.

## 5. Vitamin D analogs as potential anti-hypertensive agents?

Hypertension is a major risk factor for heart attack, stroke, myocardial infarction, congestive heart failure, progressive atherosclerosis and renal failure. As a major pathogenic contributor to hypertension, the RAS has been an important drug target for therapeutic intervention of hypertension, with ACE inhibitors and Ang II receptor antagonists being among the most popular anti-hypertensive drugs [45]. As high-renin hypertension accounts for 10–20% of the patient population with essential hypertension, specific inhibitors for renin production are of significant therapeutic values. Such inhibitors, in theory, can be used alone or in combination with ACE inhibitors or Ang II receptor antagonists. Patients with high-renin hypertension generally have higher blood pressure [46] and tend to have a more active sympathetic nervous system [47], thus renin inhibitors may also be used with sympatholytic agents such as the β-blockers. In spite of past efforts to develop peptide renin inhibitors [48–50], high toxicity of these compounds made them little use for administration to humans.

The finding that $1,25(OH)_2D_3$ suppresses renin biosynthesis has raised the possibility to develop Vitamin D analogs into renin inhibitors for therapeutic purposes. With a large number of low calcemic Vitamin D analogs synthesized, and some of them already approved for clinical uses such as treatment of secondary hyperparathyroidism [51,52], such possibility is not unrealistic. In theory, Vitamin D analogs with equal or better potency but less calcemic effects than $1,25(OH)_2D_3$ are good candidates.

To search for such candidates, we have set to screen Vitamin D analog compounds using the As4.1 cells stably transfected with human VDR by Northern blot and luciferase reporter assays. Interestingly, of the nine compounds we first screened, only the two Gemini compounds, which have double side chains at the carbon 20 position [53], displayed renin-suppressing activity equal or better than that of $1,25(OH)_2D_3$, whereas all other Vitamin D

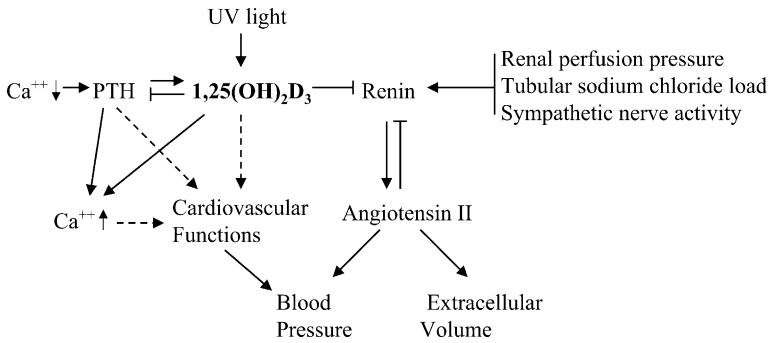


Fig. 2. Interaction of the Vitamin D endocrine system and the renin–angiotensin system. $1,25(OH)_2D_3$ feedback regulates the production of parathyroid hormone and suppresses renin biosynthesis. The latter may serve to antagonize renin production induced by other physiological factors. Renin is also feedback suppressed by Ang II. Ultraviolet light influences blood pressure via $1,25(OH)_2D_3$. $1,25(OH)_2D_3$, PTH and calcium may also directly affect the cardiovascular functions (dashed lines), as suggested by other studies. PTH, parathyroid hormone.

analogs had little or much less inhibitory activity. Continued screening of 11 more Gemini compounds identified six more candidates. The reason why the double-side chain Gemini compounds possess more potent activity in renin inhibition than other Vitamin D analogs or even $1,25(OH)_2D_3$ itself remains to be explored. Animal studies to test the in vivo efficacy of these Gemini compounds are under way in our laboratory. Preliminary data show that the analogs can significantly inhibit renin expression in vivo.

## 6. Conclusion

Studies in the last few years have presented convincing evidence that the Vitamin D endocrine system plays multiple physiological roles. The discovery that $1,25(OH)_2D_3$ suppresses renin gene expression helps explain, at least in part, the inverse relationship between Vitamin D and blood pressure observed previously. Fig. 2 outlines the interaction between the Vitamin D endocrine system and the RAS in the regulation of calcium, electrolytes, volume and blood pressure homeostasis. It is speculated that, under normal physiological conditions, in addition to maintaining the blood calcium concentration, $1,25(OH)_2D_3$ antagonizes other renin-stimulating factors to maintain an appropriate renin level in the body. The counter-balance of $1,25(OH)_2D_3$ may be crucial for preventing the detrimental over-activation of the RAS. Therefore, Vitamin D-deficiency may increase the risk of hypertension, and Vitamin D supplement may be beneficial to the cardiovascular system. Finally, low calcemic Vitamin D analogs may open a new era for the long-sought therapeutic renin inhibitors and potentially offer a new class of anti-hypertensive drugs.

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