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Vitamin D deficiency independent of hypocalcemia elevates blood pressure in rats

Flora Sundersingh, Lori A. Plum, Hector F. DeLuca*

Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, USA

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ABSTRACT

Essential hypertension is a polygenic disorder with a complex and multifactorial nature. Although no single gene is responsible, multiple genes provide incremental contributions to this disorder. Vitamin D is a primary regulator of calcium homeostasis. Epidemiological and clinical studies appear to point to a role for vitamin D in hypertension but direct experimental evidence is lacking. Sprague–Dawley rats were made vitamin D deficient by feeding a purified vitamin D-deficient diet and eliminating all sources of ultraviolet light. Vitamin D deficiency was confirmed by very low serum calcium levels. Blood pressure was measured in conscious rats non-invasively with a volume pressure recording system. Vitamin D deficiency results in elevated blood pressures independent of serum calcium concentration. The administration of 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) and a less calcemic analog, 2-methylene-19-nor-20(S)-1α–hydroxyl-bishomopregnacalciferol (2MbisP) significantly reduced blood pressure in these rats. Thus, vitamin D status is one of the determining factors regulating blood pressure.

1. Introduction

Essential hypertension (EH) is a polygenic disorder and involves environmental, demographic, vascular and neuroendocrine factors. Because of its complex and multifactorial nature, genetic determinants of EH remain mostly unknown with no single gene playing a major role. EH is rather the result of incremental contributions of multiple genes.

Vitamin D is a primary regulator of calcium homeostasis [1]. The first effect of vitamin D supplementation on blood pressure (BP) in rats was observed decades ago [2]. Further epidemiological and clinical studies established an inverse relationship between vitamin D and blood pressure [3]. Additionally, vitamin D analogs have been reported to reduce blood pressure in patients with EH [4,5]. However, a clear demonstration that D deficiency actually results in hypertension is not available [6].

We have now found that vitamin D deficiency in rats, as confirmed by severe hypocalcemia, results in hypertension and that this results from a specific lack of vitamin D and not a consequence of resulting hypocalcemia. This hypertension can be corrected by 1,25-$(OH)_2D_3$ or an analog.

2. Material and methods

2.1. Vitamin D analogs

1,25-$(OH)_2D_3$) and 2MbisP were synthesized by and purchased from Sigma Aldrich Fine Chemicals (Madison, WI). 1,25-$(OH)_2D_3$ and 2MbisP were quantitated by UV absorption in ethanol $\varepsilon^{265} = 18{,}200\ M^{-1}\ cm^{-1}$ and $\varepsilon^{252} = 42{,}000\ M^{-1}\ cm^{-1}$ respectively [7]. For intraperitoneal injection (*ip*) of vitamin D, compounds were dissolved in vehicle (5% ethanol, 95% propylene glycol) and 0.1 ml was injected daily for 3 days.

2.2. Rats

All animal experiments were conducted in accordance with the University of Wisconsin IACUC. Sprague–Dawley weanling rats were obtained from Harlan Laboratories (Indianapolis, IN) and maintained on a vitamin D-deficient diet, containing 0.47% calcium and 0.3% phosphorus (Pi) supplemented 3 times a week as described earlier

Abbreviations: 1,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3 or calcitriol; 2MbisP, 2-methylene-19-nor-20(S)-1–hydroxybishomopregnacalciferol; SBP and DBP, BP, systolic and diastolic blood pressure; MAP, mean arterial pressure; RAS, renin-angiotensin-system; ECM, extracellular matrix.

* Corresponding author. Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706-1544, USA.
E-mail address: deluca@biochem.wisc.edu (H.F. DeLuca).

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[8]. This diet was modified by increasing the calcium content to 2% by the addition of calcium carbonate and with the addition of lactose (to 10%). These substitutions were made at the expense of the glucose monohydrate (cerelose, rescue diet) [8]. Rats were housed in hanging wire cages and maintained on a 12 h light/dark cycle. Rats fed the vitamin D-deficient diet were maintained in a room with incandescent lighting, and all potential sources of ultraviolet light and vitamin D were excluded. Severe hypocalcemia was used to confirm vitamin D depletion. For the blood pressure study, 6–8 month old rats of both sexes were used, and since no gender differences were noted, the data were pooled. Serum calcium concentration was determined as previously described [9].

2.3. BP measurements

BP was measured in conscious animals non-invasively by determining the tail blood volume with a volume pressure recording (VPR) sensor and an occlusion tail-cuff (CODA System, Kent Scientific, Torrington, CT). Rats were conditioned to the procedure for one week by measuring BP daily; BP recordings were made 3 days/wk. On the data collection day, 15 BP measurements were made for each rat. The average of a set of readings was used to determine systolic BP (SBP), diastolic BP (DBP), and mean arterial pressure (MAP) for each rat. The effect of vitamin D analogs on BP in rats was studied in 8–12 month old rats by measuring BP before and 24 h post the third daily injection of 100 μl of vehicle (5% ethanol, 95% propylene glycol) (n = 8), 50 ng of 1,25-$(OH)_2D_3$ (n = 8) or 100 μg (n = 5) – 200 μg (n = 4) of 2MbisP in 100 μl of 5% ethanol, 95% propylene glycol. The data on BP in 100 μg–200 μg 2MbisP treated rats were combined in one group (as 200 μg, n = 9) for statistical purposes.

3. Results and discussion

3.1. Vitamin D-deficient rats have elevated BP

Vitamin D-deficient, 8-month old rats had significantly elevated BP ($p < 0.05$) compared to vitamin D sufficient rats: systolic (175 ± 27 vs. 139 ± 24 mmHg), diastolic (135 ± 26 vs. 107 ± 26 mmHg) and MAP (148 ± 26 vs. 117 ± 25 mmHg) (Fig. 1).

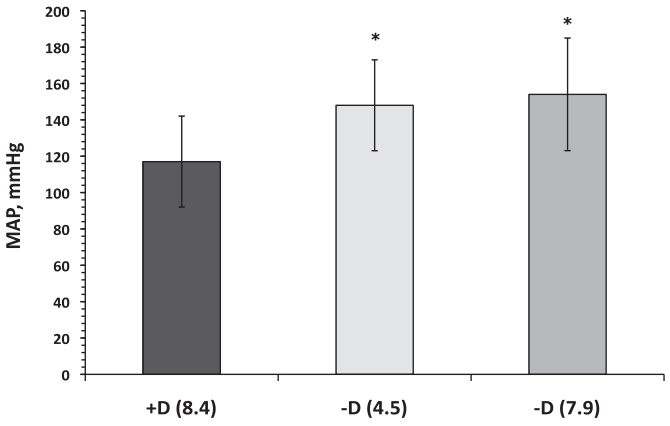

Fig. 1. Mean arterial pressure (MAP) in vitamin D-sufficient (+D) rats with normal serum Ca^{2+} (n = 24), vitamin D-deficient (–D) rats with low serum Ca^{2+} (n = 28) and vitamin D-deficient (–D) rats with normalized serum Ca^{2+} (n = 11). The average serum Ca^{2+} level in mg% for each group of rats is shown in parenthesis. The MAP of vitamin D-deficient rats was significantly different from the MAP of vitamin D-sufficient rats by t-test (*$p < 0.05$), while the MAP from rats on the vitamin D-deficient diet containing 2% calcium and 10% lactose were not different from that of the rats on the 0.47% calcium vitamin D-deficient diet.

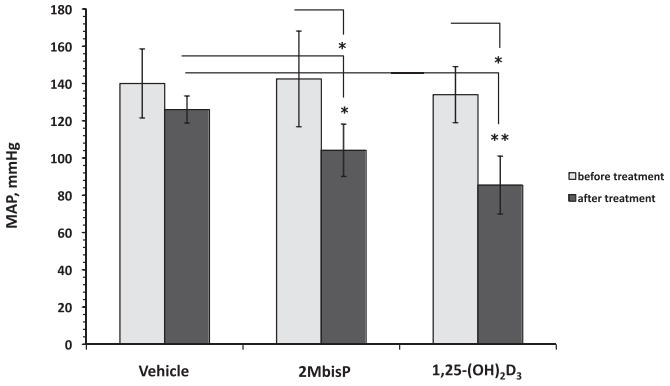

Fig. 2. Mean arterial pressure (MAP) in vitamin D-deficient (–D) rats before and 24 h after the third daily *ip* injection of 100 μl of vehicle (5% ethanol, 95% propylene glycol) (n = 8) or 200 μg 2MbisP (n = 9) or 50 ng 1,25-$(OH)_2D_3$ (n = 8) both in 100 μl of 5% ethanol, 95% propylene glycol. Significantly different from vehicle-treated rats by t-test (*$p < 0.005$; **$p < 0.00005$).

However, the vitamin D-deficient rats were severely hypocalcemic (4.5 mg/dl). To determine if the elevated BP in vitamin D-deficient rats is the result of hypocalcemia or absence of vitamin D, rats were fed the 2% calcium/10% lactose diet that increased their serum calcium level to 7.9 mg/dl (Fig. 1). Nevertheless MAP (as well as SBP and DBP) remained elevated ($p < 0.05$) (Fig. 1).

3.2. 1α,25-$(OH)_2D_3$ and a low calcemic analog 2MbisP reduce blood pressure in vitamin D-deficient rats

Treatment of vitamin D deficient rats with 150 ng/kg/day 1,25-$(OH)_2D_3$ significantly decreased SBP, DBP and MAP BP (Fig. 2). However, treatment of rats with 1.5–3.3 μg/kg/day, the 2MbisP analog did not significantly change BP, but 300–600 μg/kg/day 2MbisP significantly decreased SBP, DBP and MAP pressure, but the effect was less pronounced than for 1,25-$(OH)_2D_3$ (Fig. 2). Treatment with the highest dose of 2MbisP only slightly changed serum calcium level while 2000 times lower dose of 1,25-$(OH)_2D_3$ significantly increased their serum calcium level (Fig. 3). However, all calcium levels were either low or in the normal range.

The results presented here clearly demonstrate that vitamin D deficiency results in elevated BP. However, vitamin D deficiency causes a dramatic fall in serum calcium concentration (Fig. 1). To decide whether the elevated blood pressure in vitamin D deficiency resulted from the hypocalcemia found in the deficiency or whether it was a lack of vitamin D itself, we increased serum

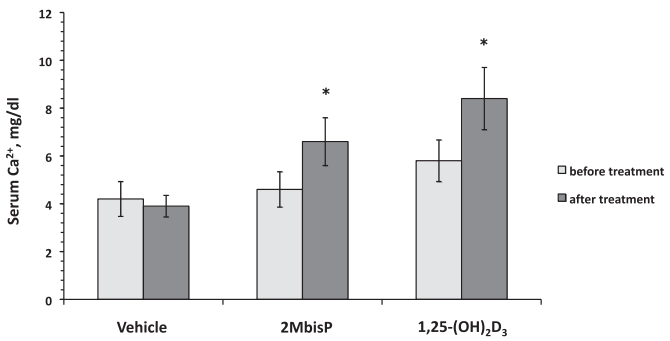

Fig. 3. Serum Ca^{2+} level in vitamin D-deficient (–D) rats before and 24 h after the third daily *ip* injection of 100 μl of vehicle (5% ethanol, 95% propylene glycol) (n = 8) or 200 μg 2MbisP (n = 9) or 50 ng 1,25-$(OH)_2D_3$ (n = 8) both in 100 μl of 5% ethanol, 95% propylene glycol. Significantly different by t-test (*$p < 0.0005$).

calcium in the vitamin D-deficient rats by feeding a high calcium lactose containing diet (rescue diet). Clearly the elevation of serum calcium in the absence of vitamin D did not cause a reduction in BP (Fig. 1). Further, the administration of the active form of vitamin D suppresses in the elevated blood pressure of deficiency, while calcium remained in the normal range. The non-calcemic analog, 2MbisP, caused a reduction in BP in animals that remained hypocalcemic. Although we have attempted to determine the basis of the reduction of BP by 1,25-$(OH)_2D_3$ by microarray analysis, we were unable to identify a gene expression basis for these results. Thus, the mechanism whereby 1,25-$(OH)_2D_3$ suppresses BP remains unknown. However, the results presented here strongly suggest that vitamin D deficiency can be a significant factor in hypertension.

Conflict of interest

The authors have declared no conflicts of interest.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.069.

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