Injection of 1,25-(OH)₂ Vitamin D₃ Enhances Resistance Artery Contractile Properties

Richard D. Bukoski, Dabao Wang, D. Wolfe Wagman

The hypothesis that 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂ vitamin D₃] modulates vascular smooth muscle contractile function was tested. 1,25-(OH)₂ vitamin D₃ (50 ng/day) was administered by intraperitoneal injection over a 3-day period to 13–15-week-old male spontaneously hypertensive and Wistar-Kyoto normotensive rats. On the fourth day, serum was prepared and contractile force generation of isolated mesenteric resistance arteries was examined. Treatment with 1,25-(OH)₂ vitamin D₃ approximately doubled serum levels of the hormone and increased ionized and total serum Ca²⁺ and phosphate by 5–10%. No effect on blood pressure was detected. 1,25-(OH)₂ vitamin D₃ injection in both strains enhanced maximal stress generation to norepinephrine and serotonin by 30–40%, with no effect on apparent sensitivity of the vessels to the agonists. To assess the effect of a maneuver that elevates serum ionized Ca²⁺ without the addition of exogenous hormone, maximal stress generation was examined in resistance arteries isolated from rats fed diets containing 0.5% or 2% calcium over a 6–7-week period. Maximal stress generation in response to norepinephrine was greater in vessels from rats of both strains maintained on 0.5% calcium. It is concluded that 72-hour in vivo treatment with 1,25-(OH)₂ vitamin D₃ increases contractile force-generating capacity of resistance arteries without affecting blood pressure. It is proposed that this action of 1,25-(OH)₂ vitamin D₃ is the result of a direct action of the hormone on the vascular wall. (Hypertension 1990;16:523–531)

The calcium-regulating hormone, 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂ vitamin D₃], works in concert with parathyroid hormone (PTH) and calcitonin to maintain serum ionized Ca²⁺ within a narrow concentration range. It has been proposed that disturbances in the PTH–vitamin D axis play a causal role in essential hypertension because of the state of relative Ca²⁺ deficiency that has been epidemiologically linked with this disorder. Indeed, 1,25-(OH)₂ vitamin D₃ has been reported to be elevated in serum of humans with low renin essential hypertension. Although the mechanisms by which 1,25-(OH)₂ vitamin D₃ might influence blood pressure are as yet unknown, there is growing evidence that this hormone is a modulator of cardiovascular function. Receptors that are specific for 1,25-(OH)₂ vitamin D₃ have been described in cultured vascular smooth muscle cells. Furthermore, it has been reported that 1,25-(OH)₂ vitamin D₃ acutely increases the whole animal pressor response to infused catecholamines and may selectively constrict regional circulations. Although these acute actions of the hormone have been reported in intact animals, the vascular effects of longer term administration have not been examined. The present series of experiments test the hypothesis that in vivo administration of 1,25-(OH)₂ vitamin D₃ acutely increases the whole animal pressor response to infused catecholamines and may selectively constrict regional circulations. Although these acute actions of the hormone have been reported in intact animals, the vascular effects of longer term administration have not been examined. The present series of experiments test the hypothesis that in vivo administration of 1,25-(OH)₂ vitamin D₃ modifies contractile properties of subsequently isolated vascular smooth muscle. Both spontaneously hypertensive (SHR) and Wister-Kyoto (WKY) normotensive rats were examined to determine whether 1,25-(OH)₂ vitamin D₃ has differential effects on the hypertensive animal.

Methods

Animals—Injection Studies

Male SHR and WKY rats (Charles River Breeding Farms, Wilmington, Mass.) were used between 13
and 15 weeks of age. The animals were maintained in a humidified temperature- and light-controlled environment and given free access to rodent chow (5001, Ralston Purina Co., St. Louis, Mo.) and tap water. Several days after arrival in our animal quarters and before entry into the study, the systolic blood pressure of each animal was determined using the pneumatic tail-cuff method (Narco Biosystems, Houston, Tex.).

For 3 consecutive days, between 9:00 and 9:30 AM, the animals were given an intraperitoneal injection of either 50 ng 1,25-(OH)2 vitamin D3 (Hoffman-LaRoche, Nutley, N.J.) or vehicle (50 μl propylene glycol + 200 μl isotonic saline). On the afternoon of the third day, the blood pressure of each rat was redetermined. On the fourth day, the animal was anesthetized with ether and exsanguinated via cardiac puncture. The blood was collected into evacuated tubes, allowed to clot on ice for 20 minutes, and then centrifuged at 10,000 rpm for 20 minutes to obtain a serum fraction. Mesenteric arteries were then isolated for measurement of isometric force development.

**Animals—Feeding Studies**

Male SHR and WKY rats were obtained from Charles River at 4 weeks of age and randomly placed on purified diets containing 0.5% or 2% calcium, given as the carbonate salt. These diets were formulated by Teklad (Madison, Wis.) and have previously been used by our laboratory.11 Other key nutrient levels were phosphate 0.84%, potassium 1%, sodium 0.45%, protein (casein) 20%, and fiber 5%. Between 10 and 11 weeks of age, the rats were killed and mesenteric resistance arteries were isolated and prepared for the measurement of isometric contraction as described in the following section.

**Mechanical Measurements**

At the time of death, a loop of intestine 20–40 mm distal to the pylorus was removed and placed in cold physiological salt solution (PSS) of the following composition in mM: NaCl 130, KCl 4.7, MgSO4·7H2O 1.15, NaHCO3 15, Na2HPO4 1.15; KH2PO4 1.25, CaCl2 1.25, and glucose 5.0 (pH 7.4 when gassed with 95% O2–5% CO2 mixture). Branch II or III resistance arteries were then dissected away from the omentum and adhering fat layers and mounted in the chamber of a dual channel myograph (Midori Cascade, Aloha, Ore.) equipped with Kulite-BG-10 (Kulite Semiconductors, Leonia, N.J.) force transducers.

While viewed in the field of a ×40 objective (Nikon, Tokyo), slack on the vessel was taken up by passive stretch and the volume of the medial layer calculated from values of diameter, axial length, and wall thickness determined morphometrically with the vessel set to its initial length. The vessel was then stretched to 90% of the length that it would have with an intraluminal pressure of 100 mm Hg, as described by Mulvany and Halpern,12 and the medial thickness at this length was calculated from values of the diameter of the segment, its axial length, and the media volume, which was assumed to remain constant.

After making the morphometric measurements and standardizing the length of the vessel, the contractile response of each vessel to a challenge with 100 mM KCl (NaCl substituted) was determined three times, followed by two challenges with 100 mM KCl/10 μM norepinephrine. The contractile response of each vessel to the cumulative addition of norepinephrine and serotonin was then determined as described previously.11 The apparent sensitivity of each vessel to norepinephrine and serotonin was assessed using the concentration of the agonist that elicited 50% of the maximal response (EC50). The EC50 values were determined from plots of the percent maximal response versus the log molar concentration of the agonist. The force generated by each vessel was normalized to cross-sectional area of the vessel and is reported as active stress (mN/mm2). Cross-sectional area is the product of media thickness and axial length of the vessel.

**Serum Determinations**

Sera were analyzed for ionized Ca2+ using a Ca2+-specific electrode (Radiometer, Copenhagen). Total Ca2+ and inorganic phosphate were determined colorimetrically using the COBAS-Bio assay system (Roche Diagnostics, Nutley, N.J.). Serum 1,25-(OH)2 vitamin D3 was determined using a radioreceptor assay (INCSTAR Corp., Stillwater, Minn.).

**Statistical Analysis**

All values are reported as the mean±SEM. Differences between strains and the effect of the 1,25-(OH)2 vitamin D3 treatment were assessed using two-way analysis of variance (ANOVA). The effect of multiple concentrations of the agonists were assessed using a repeated-measures ANOVA. The SYSTAT software package (SYSTAT, Evanston, Ill.) was used in each case. Results were considered to be significantly different at a value of p<0.05.

**Results**

The rats used in the injection study were matched in terms of age. Table 1 shows that body weight of both the 1,25-(OH)2 vitamin D3- and vehicle-treated WKY rats increased over the 3-day injection period. In contrast, the SHR in both the treated and vehicle control groups failed to gain weight. This difference in weight gain was presumably the result of decreased food consumption caused by the stress of handling during this period. In contrast to the differential effect of the protocol on body weight, no effect of 1,25-(OH)2 vitamin D3 or vehicle on blood pressure was observed in rats of either strain, although as expected, SHR pressure was greater than that of WKY rats (Table 2).

Basal levels of serum Ca2+ and phosphate were significantly elevated in the WKY rats compared with...
The same pattern of response was observed for the rats that received only vehicle (Figures 1 and 2). WKY rats generated more active stress than those contractile response of the vessels to serotonin (Figures 3 and 4) and to a single challenge with 100 mM norepinephrine (Table 6). In addition, no differences in pD2 values for norepinephrine or serotonin were detected between SHR and WKY rats.

Table 1. Effect of 1,25-Dihydroxyvitamin D3 Injection on Body Weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>290±8.1</td>
<td>281±9.8</td>
</tr>
<tr>
<td>(g)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>WKY</td>
<td>244±7.3</td>
<td>272±11.8</td>
</tr>
<tr>
<td>(g)</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

1,25-Dihydroxyvitamin D3 or vehicle was injected over a 3-day period as described in the text. Body weight was determined on the day before the rat was used in the experiment before the injections were begun. Values are mean±SEM. No effect of 1,25-dihydroxyvitamin D3 versus vehicle was detected in either strain.

*Indicates a significant strain difference at p<0.001.
†Indicates a significant difference between periods before and after treatment at p<0.001.

SHR, but no differences in basal levels of 1,25-(OH)2 vitamin D3 were detected. After the series of 1,25-(OH)2 vitamin D3 injections, the level of this hormone was elevated in rats of both strains (Table 3). This increase was accompanied by a slight (5%) increase in the level of serum ionized total serum Ca2+ as well as in total serum phosphate.

The diameter of the vessels that were examined did not differ significantly either across strain lines or among the treatment groups (Table 4). The diameter of the vessels ranged from approximately 220 to 260 μm, and although the thickness of the media tended to be greater in vessels of the SHR, no significant difference was detected. There was, however, a significant difference in the wall/lumen ratio with values for SHR being greater (Table 4). No effect of the 1,25-(OH)2 vitamin D3 injections on wall thickness was detected.

When the contractile response of the resistance arteries to norepinephrine was examined, those isolated from 1,25-(OH)2 vitamin D3-treated SHR and WKY rats generated more active stress than those from rats that received only vehicle (Figures 1 and 2). The same pattern of response was observed for the contractile response of the vessels to serotonin (Figures 3 and 4) and to a single challenge with 100 mM KCl and 10 μM norepinephrine (Table 5). In contrast to the level of force development, administra-

Table 2. Effect of 1,25-Dihydroxyvitamin D3 on Blood Pressure

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>160±2.6</td>
<td>157±4.0</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>WKY</td>
<td>106±1.7*</td>
<td>110±1.4*</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

1,25-Dihydroxyvitamin D3 or vehicle was injected over a 3-day period as described in the text. Systolic blood pressure was determined the day before the animal was used in the experiment before beginning the injections. Values are mean±SEM. No effect of 1,25-dihydroxyvitamin D3 versus vehicle was detected in either strain.

*Indicates a significant strain difference at p<0.05.
FIGURE 1. Line graphs showing effect of cumulative addition of norepinephrine (NE) to mesenteric resistance arteries isolated from spontaneously hypertensive rats (SHR). Repeated-measures analysis of variance indicated that vessels from 1,25-dihydroxyvitamin D₃-treated rats were significantly different at p<0.05 when active stress (top panel) was examined, although no effect on percent maximal response (bottom panel) was detected. All values are mean±SEM and n=9 or 10 for each point.

Discussion

Evidence from epidemiological surveys and human clinical trials suggests that disturbances in whole animal Ca²⁺ metabolism play a role in the hypertensive process.¹²,²⁴ It has been suggested that the calcitropic hormones, 1,25-(OH)₂ vitamin D₃, PTH, and calcitonin, which serve to regulate serum ionized Ca²⁺, have direct vascular actions that might result in a hyperreactive state of the blood vessel wall.² The present study was designed to examine the effect of intraperitoneal administration of 1,25-(OH)₂ vitamin D₃ on the contractile properties of subsequently isolated blood vessels.

The animals used in this study were 13–15-week-old male SHR and WKY rats. The SHR model was chosen because of numerous disturbances in whole animal and cell Ca²⁺ metabolism. These include depressed serum ionized Ca²⁺ levels,¹³ hypercalciuria,¹³,¹⁴ defective Ca²⁺ transport by intestinal epithelia,¹⁵ elevated levels of free ionized Ca²⁺ in platelets and cultured vascular smooth muscle cells,¹⁶–¹⁸ and enhanced sensitivity of isolated vascular smooth muscle to extracellular Ca²⁺.¹⁹–²¹ In agreement with these observations, SHR that were studied exhibited suppressed levels of serum ionized Ca²⁺. Furthermore, basal levels of serum 1,25-(OH)₂ vitamin D₃ were not different between the strains, which is characteristic of these animals at 12

were not determined for the rats used in this part of the study, a previous report from our laboratory showed that the 2% calcium diet caused a 5–10% increase in serum ionized calcium compared with the 0.5% diet in both strains.¹¹ When contractile force-generating capacity of mesenteric resistance arteries isolated from these rats was examined, it was observed that vessels isolated from rats fed diets with 2% calcium developed significantly less force than those from rats fed diets containing the lower level of calcium (Table 7).
weeks of age. The other parameters were not specifically examined.

The most important result of this study is the observation that administration of 1,25-(OH)₂ vitamin D₃ at concentrations that result in modest physiological increases in serum levels of the hormone enhances contractile force-generating capacity of isolated mesenteric resistance arteries. In addition, the observation that the response to 1,25-(OH)₂ vitamin D₃ was similar in both SHR and WKY rats indicates that the response is a general property of vascular smooth muscle and is not specific for the hypertensive animal.

These results join a growing list of findings that are consistent with the hypothesis that 1,25-(OH)₂ vitamin D₃ is a modulator of vascular function. For example, it has been demonstrated that receptors specific for 1,25-(OH)₂ vitamin D₃ are present in vascular smooth muscle cells and that the hormone is capable of altering Ca²⁺ uptake by cultured vascular myocytes. In addition, at least two laboratories have demonstrated that 1,25-(OH)₂ vitamin D₃ can increase the rate of growth of vascular myocytes in culture, whereas a third reports an inhibitory effect of the hormone under different conditions. It has also been observed that acute administration of 1,25-(OH)₂ vitamin D₃ potentiates subsequent pressor responses to norepinephrine in conscious SHR and enhances the sensitivity of isolated mesenteric resistance arteries to both serotonin and norepinephrine.

A key question arising from the present study concerns the mechanism of the inotropic effect of 1,25-(OH)₂ vitamin D₃ on the resistance artery. The fact that the effect was detected in a standardized salt solution at least 2 hours after the vessel was isolated from the animal suggests that an intrinsic change in the vessel wall occurs in response to the hormone. Furthermore, the finding that the elevated contractile responses of vessels isolated from 1,25-(OH)₂ vitamin D₃-treated rats were observed with several agonists indicates that the hormone exerts its actions primarily through postreceptor events. This conclu-
**Hypertension** Vol 16, No 5, November 1990

**TABLE 5. Effect of 1,25-Dihydroxyvitamin D3 on Active Stress Response**

<table>
<thead>
<tr>
<th>Group</th>
<th>NE (mN/mm²)</th>
<th>5-HT (mN/mm²)</th>
<th>KNE (mN/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR-veh</td>
<td>131±20 (10)</td>
<td>121±19 (10)</td>
<td>125±16 (10)</td>
</tr>
<tr>
<td>SHR-1,25D3</td>
<td>207±26* (10)</td>
<td>195±27* (10)</td>
<td>195±25* (10)</td>
</tr>
<tr>
<td>WKY-veh</td>
<td>157±19 (9)</td>
<td>128±14 (9)</td>
<td>168±18 (9)</td>
</tr>
<tr>
<td>WKY-1,25D3</td>
<td>215±28* (10)</td>
<td>192±30* (10)</td>
<td>221±30* (10)</td>
</tr>
</tbody>
</table>

Maximal active stress response of mesenteric resistance arteries isolated from spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats injected with either 1,25-dihydroxyvitamin D3 (1,25D3) or vehicle (veh). Agonists used were norepinephrine (NE), serotonin (5-HT), and 100 mM KCl/10 μM norepinephrine (KNE). Values are mean±SEM. No strain differences were detected.

*Indicates a significant effect of the 1,25D3 injection at a p<0.05.

**FIGURE 3.** Line graphs showing effect of cumulative addition of serotonin (5-HT) to mesenteric resistance arteries isolated from spontaneously hypertensive rats (SHR). Repeated-measures analysis of variance indicated that vessels from 1,25-dihydroxyvitamin D3-treated rats were significantly different at p<0.05 when active stress (top panel) was examined, although no effect on percent maximal response (bottom panel) was detected. All values are mean±SEM and n=9 or 10 for each point.

Maximal active stress response of mesenteric resistance arteries isolated from spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats injected with either 1,25-dihydroxyvitamin D3 (1,25D3) or vehicle (veh). Agonists used were norepinephrine (NE), serotonin (5-HT), and 100 mM KCl/10 μM norepinephrine (KNE). Values are mean±SEM. No strain differences were detected.

*Indicates a significant effect of the 1,25D3 injection at a p<0.05.

The hormone had no effect on the apparent sensitivity of the vessels to these agonists.

With respect to the latter observation, however, it should be noted that the present experiments were not carried out in the presence of an inhibitor of neuronal uptake, such as cocaine, or in chemically sympathectomized vessels. Thus, although we have demonstrated that the sensitivity of the intact vessel is not altered by 1,25-(OH)2 vitamin D3, the possibility that a change in smooth muscle sensitivity that is masked by altered activity of the neuronal pump (as has been reported in blood vessels of SHR25-27 and humans with essential hypertension28) cannot be ruled out at the present time.

As noted above, results of experiments carried out using cultured vascular myocytes are consistent with the hypothesis that 1,25-(OH)2 vitamin D3 acts directly on the vascular smooth muscle cell. For exam-
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FIGURE 4. Line graphs showing effect of cumulative addition of serotonin (5-HT) to mesenteric resistance arteries isolated from Wistar-Kyoto (WKY) rats. Repeated-measures analysis of variance indicated that vessels from 1,25-dihydroxyvitamin D3-treated rats were significantly different at p<0.05 when active stress (top panel) was examined, although no effect on percent maximal response (bottom panel) was detected. All values are mean±SEM and n=9 or 10 for each point.

ple, it has been demonstrated that 1,25-(OH)2 vitamin D3-enhanced 45Ca uptake is blocked by inhibitors of protein synthesis. It is tempting to speculate that this action of 1,25-(OH)2 vitamin D3 is related to its inotropic effects on the vitamin D-dependent calcium binding proteins that are present in a variety of tissues.

It is also known that 1,25-(OH)2 vitamin D3 modulates the growth of vascular smooth muscle cells in culture. Because medial thickness is a factor in the calculation of active wall stress, it is possible that the 1,25-(OH)2 vitamin D3-induced changes reflect a change in the thickness of the wall. No significant effect of the hormone injections on medial thickness was observed, however, suggesting that the inotropic actions of 1,25-(OH)2 vitamin D3 are independent of its growth-stimulatory properties.

Given the importance of the endothelium as a modulator of vascular function, its potential role as a mediator of the vascular actions of 1,25-(OH)2 vitamin D3 must also be considered. Along these lines it is noteworthy that Merke and coworkers have demonstrated the presence of 1,25-(OH)2 vitamin D3 receptors on cultured endothelial cells. Furthermore, our group has recently observed that endothelium-dependent relaxation of resistance arteries is altered in the parathyroidectomized SHR, suggesting that the endothelium is a target for hormones of the PTH–vitamin D axis. If the endothelium is involved in mediating the 1,25-(OH)2 vitamin D3-induced changes, then it is likely that the hormone either suppresses the release of endothelium-derived relaxing factor or promotes the release of an endothelium-derived contracting factor, such as endothelin. Additional studies need to be carried out to assess the potential contribution of the endothelium.

The observation that there were no differences in the apparent sensitivity to norepinephrine or serotonin contrasts with our previous finding that acute administration of 1,25-(OH)2 vitamin D3 to isolated vessels causes an increase in sensitivity to these agonists. Possible explanations for the differences between the acute and long-term responses include the fact that in the intact animal, serum parameters
other than 1,25-(OH)_2 vitamin D_3 are altered and that different periods of exposure were in effect. Furthermore, the possibility that different receptors for 1,25-(OH)_2 vitamin D_3 are involved must also be considered. For example, although the 3-day effects of 1,25-(OH)_2 vitamin D_3 on force generation may be mediated by the classic steroid receptor, the acute actions of the hormone on sensitivity to contractile agonists may be mediated by another type of membrane-associated receptor.36-37

The finding that SHR failed to gain weight during the 3-day injection period raises the possibility that the vascular effects of 1,25-(OH)_2 vitamin D_3 are the result of an effect on general growth and vigor of the animals. This seems unlikely, however, given the fact that contractile force generation was enhanced to similar degrees in rats of both strains and only SHR failed to gain weight. It is also possible that the inotropic effect of 1,25-(OH)_2 vitamin D_3, given over a 3-day period, does not increase systolic blood pressure as measured using the tail-cuff method in either SHR or WKY rats (Table 2) even though intrinsic force-generating ability of the resistance arteries is increased. These observations suggest, among other things, that an increase in resistance artery force-generating capacity alone is insufficient to raise blood pressure in the rat. Given the redundant systems that are present in the mammal for controlling blood pressure (i.e., nervous reflexes and volume regulatory systems)39 the lack of effect of the short-term administration of the hormone is not surprising. It is possible that administration of the steroid over a longer period of time would be accompanied by escape from overriding blood pressure control mechanisms or by structural changes, which would serve to increase peripheral resistance. In addition, it is possible that in vivo pressor responses to contractile agonists or stress might be elevated while basal blood pressure remains unchanged. These possibilities remain to be explored.

Acknowledgments

We are grateful for the technical assistance of Julie Brown, Ann-Marie Dolney, and Jamie DeMerritt and the editorial skills of Molly Reusser.

References

Vitamin D, and Vascular Contractility

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Key Words: vascular resistance • essential hypertension • vitamin D3
Injection of 1,25-(OH)2 vitamin D3 enhances resistance artery contractile properties.
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Hypertension. 1990;16:523-531
doi: 10.1161/01.HYP.16.5.523

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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