Enhancement of Vasoconstrictor Response by a Noncalcemic Analogue of Vitamin D₃

Tatsuo Shimosawa, Katsuyuki Ando, and Toshiro Fujita

To clarify the effects of active vitamin D₃ on pressor and vascular responses to vasoconstrictor substances, we studied pressor responses to the intravenous injection of norepinephrine or angiotensin II (Ang II) and vasoconstrictor responses to norepinephrine. Sprague-Dawley rats were given 1,25-dihydroxyvitamin D₃ subcutaneously (200 ng/kg per day) for 14 days. The administration of 1,25-dihydroxyvitamin D₃ augmented the pressor responses to norepinephrine and Ang II in conscious rats and was associated with a significant increase in serum calcium concentration (11.0±0.2 mg/dl). To further clarify whether the increased pressor response to vasoconstrictors may be due to the calcemic or direct action of active vitamin D₃, we studied the effect of its noncalcemic analogue, 22-oxacalcitriol, and its inactive analogue, 24,25-dihydroxyvitamin D₃, on the pressor response to vasoconstrictors in rats. The pressor responses to norepinephrine and Ang II were apparently augmented in 22-oxacalcitriol-treated rats similarly to 1,25-dihydroxyvitamin D₃-treated rats. In contrast, the pressor responses were not affected by either 24,25-dihydroxyvitamin D₃ or the intravenous infusion of calcium chloride. In an ex vivo experiment using a mesenteric preparation, the vascular sensitivity to norepinephrine was moderately augmented in rats treated with both 22-oxacalcitriol and 1,25-dihydroxyvitamin D₃ but was not affected in rats treated with 24,25-dihydroxyvitamin D₃. The results suggest that the enhanced pressor responses to norepinephrine and Ang II could be attributed to the direct effect of active vitamin D₃ on vasculature rather than to hypercalcemia. (Hypertension 1993;21:253-258)

**Key Words** • calcitriol • norepinephrine • angiotensin II • hypertension, essential • calcium • 24,25-dihydroxyvitamin D₃

Disturbed calcium metabolism has been known as one of the factors that cause or maintain hypertension. It is still unclear, however, what the main element is in the hypertensinogenic mechanism with disturbed calcium metabolism. Recently, 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] has been studied as an important candidate. The level of 1,25(OH)₂D₃ is higher in hypertensive patients with low plasma renin activity. Recent reports have shown that, in adolescent male subjects with a strong family history of hypertension, arterial pressure was increased by salt loading concomitant with elevation of 1,25(OH)₂D₃. Moreover, 1,25(OH)₂D₃ may be intimately related to the regulation of arterial pressure; its receptor has been shown to localize in vascular smooth muscle cell. In fact, the vasoconstrictor responses to norepinephrine and serotonin were enhanced in the mesenteric arteries of 1,25(OH)₂D₃-treated spontaneously hypertensive and Wistar-Kyoto rats, and 1,25(OH)₂D₃ constricted the renal vasculature in Sprague-Dawley rats.

It is well known that the physiological action of 1,25(OH)₂D₃ is divided into two categories: calcemic and noncalcemic effects. Because changes in intracellular and extracellular calcium concentrations modulate smooth muscle contractility in both in vitro and in vivo studies, the calcemic effect of 1,25(OH)₂D₃ might in some way contribute to the increased pressor action of the active vitamin D₃. Elevation of serum calcium concentration in forearm blood flow with intrabrachial infusion of calcium not only increased basal forearm vascular resistance but also augmented the forearm vascular responses to vasoactive substances in humans. In addition to its calcemic effect, 1,25(OH)₂D₃ has noncalcemic effects, such as stimulating cell differentiation, depressing parathyroid hormone, and enhancing the immune response. We therefore should consider both noncalcemic and calcemic effects as contributing to the vasoconstrictive action of 1,25(OH)₂D₃.

There have been no previous studies on the noncalcemic action of 1,25(OH)₂D₃ on vasculature. To evaluate whether noncalcemic effects of 1,25(OH)₂D₃ may be involved in increased vasoconstrictor responses, we studied the effect of chronically administered 1,25(OH)₂D₃, its noncalcemic analogue 22-oxacalcitriol (OCT), or its inactive analogue 24,25-dihydroxyvitamin D₃ [24,25(OH)₂D₃] on the in vivo and ex vivo vascular responses to norepinephrine or angiotensin II (Ang II) in Sprague-Dawley rats.

**Methods**

**Protocol 1**

Forty 8-week-old male Sprague-Dawley rats (body weight, 200-250 g; Charles River Japan, Atsugi, Japan) were subjected to continuous infusion of 200
Eight age-matched male Sprague-Dawley rats were anesthetized as in protocol 1, and the carotid artery and jugular vein were cannulated with PE-50 polyethylene tubing. The arterial and venous catheters were tunneled to the back of the neck, filled with heparinized saline (100 units/ml), and plugged with stainless steel pins. Rats were left for 3 hours until the effect of anesthesia disappeared. The carotid arterial catheter was flushed and attached to a pressure transducer (model TP-200T, Nihon Kohden), and blood pressure and heart rate were recorded on a thermal array recorder (model WS-641G, Nihon Kohden). Carotid arterial pressure was measured as mean arterial pressure (MAP).

After basal MAP and heart rate measurement, norepinephrine (10⁻⁸, 10⁻⁷, and 0.5 x 10⁻⁶ mol/kg) or Ang II (12.5, 25, and 50 ng/kg) dissolved in 0.1 ml saline was injected into the jugular vein as a bolus. The peak values of increases in MAP were considered as the response to each dose of the vasoconstrictor. Each injection was done 15 minutes after MAP returned to basal level. After the pressor response experiment, blood samples were taken for measurement of serum calcium level.

**Protocol 2**

In twenty-three Sprague-Dawley rats, 1,25(OH)₂D₃, OCT, 24,25(OH)₂D₃, or propylene glycol was administered in the same manner as in protocol 1. Fourteen days after treatment, the rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.), and the intestinal loop containing the mesenteric artery was prepared with a modified form of Castellucci's method. The abdominal cavity was opened, the superior mesenteric artery was located, and the proximal segment was cleaned of surrounding tissue in the area of the aorta. A PE-50 polyethylene catheter was inserted distally into the main trunk of the mesenteric artery at its origin from the aorta and was tied in place. The mesenteric vascular bed was flushed with approximately 3 ml heparinized Krebs-Henseleit solution, and then the large intestine was isolated and discarded. The entire mesenteric-intestinal loop was cut and quickly connected to the perfusion apparatus. The preparations were perfused with Krebs-Henseleit solution by use of a peristaltic pump (Minipuls 2, Gilson Medical Electronics SA, Villiers-le-Bel, France) at a rate of 3 ml/min. Constituents of the solution were as follows (mmol/l): NaCl 114.5, KCl 4.6, KH₂PO₄ 1.4, MgSO₄ 2.4, CaCl₂ 2.5, NaHCO₃ 25, and glucose 5.6. The solution was continuously oxygenated with a gas mixture of 95% O₂-5% CO₂ at 37°C. A 30-minute equilibration period was allowed before each experiment was started. Norepinephrine in a dose of 5, 10, 50, or 100 μmol was injected as a bolus with a microinjector. The change of perfusion pressure was recorded by a pressure transducer (model TP-200T, Nihon Kohden) connected to a thermal array recorder (model WS-641G, Nihon Kohden). Percent change of perfusion pressure was then calculated.

**Drugs**

We used OCT as a noncalcemic analogue of 1,25(OH)₂D₃. OCT differs from 1,25(OH)₂D₃ solely by the substitution of an oxygen atom for the methylene group at carbon 22 and is a highly selective noncalcemic analogue of 1,25(OH)₂D₃. OCT is as potent as or more potent than 1,25(OH)₂D₃ in stimulating cell differentiation. OCT inhibits the preproparathyroid hormone messenger RNA levels in vivo as 1,25(OH)₂D₃ does and enhances the immune response in mice. In contrast, OCT was at least 100 times less calcemic than 1,25(OH)₂D₃ in normal rats and in mice. OCT and 1,25(OH)₂D₃, 24,25(OH)₂D₃, and OCT were a kind gift from Chugai Pharmaceutical Co., Ltd., Tokyo. Norepinephrine and Ang II were obtained from Sigma Chemical Co., St Louis, Mo.

**Statistical Methods**

All data are expressed as mean±SEM. Data were analyzed with a two-way analysis of variance, and subsequent multiple comparison was done by Scheffe's
Results

Protocol 1

Body weight, basal MAP, heart rate, and serum calcium concentrations are shown in Table 1. Among the five experimental groups, body weight, MAP, and heart rate did not differ. Serum calcium levels were significantly higher in the 1,25(OH)₂D₃ group than in the control group (p<0.01). Serum calcium levels in OCT- or 24,25(OH)₂D₃-treated rats, however, did not significantly differ from those in vehicle-treated control rats.

In vehicle-treated rats, norepinephrine injected at the doses of 10⁻⁸, 10⁻⁷, and 0.5×10⁻⁶ mol/kg increased MAP in a dose-dependent fashion (7±2, 9±1, and 18±2 mm Hg, respectively (Figure 1). 1,25(OH)₂D₃ treatment enhanced the pressor effect of norepinephrine (11±1, 20±1, and 39±1 mm Hg; p<0.01), but 24,25(OH)₂D₃ did not (4±1, 8±1, and 17±2 mm Hg; NS). Despite unchanged serum calcium levels, the increases of MAP with norepinephrine were significantly greater in OCT-treated rats than in control rats (12±1, 18±1, and 31±2 mm Hg; p<0.01). The percent decrease of heart rate was -16±14%, -28±15%, and -36±16% in the control group and -15±16%, -16±16%, and -39±15% in the 24,25(OH)₂D₃ group.

Note that the response of MAP to norepinephrine was significantly increased in both Vit D₃- and OCT-treated rats as compared with vehicle- or 24,25D₃-treated rats with calcium infusion. **p<0.01 (versus vehicle) by Scheffe’s method.

Statistical significance was defined at a value of p<0.05.

Table 2. Change in Mean Arterial Pressure and Heart Rate With Angiotensin II

<table>
<thead>
<tr>
<th>Group</th>
<th>Angiotensin II (ng/kg)</th>
<th>12.5</th>
<th>25</th>
<th>50</th>
<th>p (vs. vehicle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n=7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>4±1</td>
<td>9±2</td>
<td>15±4</td>
<td></td>
<td>...</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>-15±12</td>
<td>-29±15</td>
<td>-33±16</td>
<td></td>
<td>...</td>
</tr>
<tr>
<td>OCT (n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>7±1</td>
<td>14±1</td>
<td>25±2</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>-22±13</td>
<td>-30±14</td>
<td>-58±16</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Calcium (n=4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>9±1</td>
<td>18±2</td>
<td>29±4</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>-27±16</td>
<td>-37±20</td>
<td>-64±22</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; bpm, beats per minute; 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; OCT, 22-oxacalcitriol; 24,25(OH)₂D₃, 24,25-dihydroxyvitamin D₃. Data are mean±SEM.
caused dose-dependent increases in MAP. In CaCl2-versus saline-infused rats, there was no significant difference in pressor response to either norepinephrine (2±1 versus 2±1, 11±1 versus 10±1, and 16±2 versus 16±2 mm Hg; NS) (Figure 1) or Ang II (4±1 versus 3±1, 9±1 versus 9±1, and 15±2 versus 14±2 mm Hg; NS). The decrease of heart rate with norepinephrine or Ang II was not significantly different between the two groups (data not shown).

**Protocol 3**

Body weight [315±1, 288±5, 305±4, and 303±4 g; control, 1,25(OH)2D3, OCT, and 24,25(OH)2D3, respectively] and serum calcium level (9.6±0.1, 11.1±0.2, 9.9±0.2, and 10.0±0.2 mg/dl, respectively) showed similar tendencies to those in protocol 1. Changes of perfusion pressure with norepinephrine at 5, 10, 50, and 100 mmol/L, OCT, and 1,25(OH)2D3, respectively, were not enhanced until serum calcium levels reached 13.0±0.5 mg/dl, levels that were much higher than those in the 1,25(OH)2D3-treated rats in our study (11.0±0.2 mg/dl, p<0.01, Student’s unpaired t test). Moreover, in vitro studies with femoral artery rings from deoxycorticosterone acetate-salt and Sprague-Dawley rats revealed that high extracellular ionized calcium (2.5 mM) enhanced vasoconstriction by KC1 and norepinephrine compared with low calcium (0.25 mM). The mild increase in plasma calcium level (11.2±1.0 mg/dl), although similar to that in our study (11.0±0.2 mg/dl), did not affect pressor responses to norepinephrine and Ang II in normal subjects. Thus, the 1,25(OH)2D3 dosing used in this study may enhance pressor responses to norepinephrine and Ang II independent of the elevation of serum calcium level. In addition, the administration of 24,25(OH)2D3, an inactive vitamin D3 analogue,28 had no effect on pressor responses to norepinephrine and Ang II, suggesting that pressor enhancement by 1,25(OH)2D3 is not due to its nonspecific action.

Because of the augmentation of responses to norepinephrine by 1,25(OH)2D3, we can speculate that 1,25(OH)2D3 can modulate sympathetic tone directly, because norepinephrine release at presynaptic nerve endings depends on its intracellular calcium concentration.29 However, Baks and Hughes28 reported that vitamin D supplementation did not affect norepinephrine release or metabolism. In fact, in the present study, the changes in heart rate with infusion of norepinephrine or Ang II corresponded to those in MAP, suggesting that baroreceptor function is not affected by the treatments. 1,25(OH)2D3-induced enhancement of the...
pressor response to norepinephrine might not be attributed to changes in sympathetic tone, although the effect of active vitamin D₃ on norepinephrine release was not examined. The results of the present study suggest that increased pressor response to norepinephrine by 1,25(OH)₂D₃ may be the result of enhanced vascular smooth muscle cell contractility through nonspecific agonist-mediated receptor reaction. This hypothesis is supported by the present finding that 1,25(OH)₂D₃ could augment not only the pressor response to norepinephrine but also that to Ang II.

Norepinephrine- or Ang II-induced contraction of vascular smooth muscle cell is dependent on an increased concentration of intracellular free ionized calcium. But, in our study, OCT, which does not elevate intracellular calcium in either nongenomic17 or genomic pathways, augmented vascular responsiveness. The possible role of changes in intracellular calcium level, however, is still unknown, and further study is required.

Recent studies on vasobefective substances and endothelium lead us to conclude that endothelial function is one of the important factors in regulating vascular tone.27,28 It is known that there are 1,25(OH)₂D₃ receptors on endothelial cells.29 Although little is known about the effect of 1,25(OH)₂D₃ on endothelial function, hyperviaminosis D in rats caused endothelial degeneration.30 In contrast, a recent study by Xue et al31 indicated that 1,25(OH)₂D₃ preserved endothelial function in culture conditions. In in vitro conditions, vascular relaxation with acetylcholine is well preserved when active vitamin D₃ is added in media. Our study conditions are different from the conditions in the report of Xue et al,31 but releasing ability of endothelium-derived relaxing factor might not be the cause of the vasoconstriction observed. The apparently discrepant results may be due to different experimental conditions between our study and Xue’s. The noncalcemic effect of 1,25(OH)₂D₃ on endothelial function still remains to be determined.

Finally, because of the active vitamin D₃-induced enhancement of pressor and vasoconstrictor responses to vasoactive substances, we should consider the possible role of active vitamin D₃ on vascular hypertrophy. Evidence includes the existence of 1,25(OH)₂D₃ receptors on vascular smooth muscle cells3-5 and the 1,25(OH)₂D₃ modulation of the growth of vascular smooth muscle cells in vitro32,33 and in vivo.30 If the administration of 1,25(OH)₂D₃ and OCT in rats could cause vascular hypertrophy, it may also be involved in the increased pressor and vascular responses to norepinephrine and Ang II through the changes in vascular wall-to-lumen ratio. Further investigations are required to clarify these possibilities.

In conclusion, long-term treatment with 1,25(OH)₂D₃ in rats enhanced pressor responses to norepinephrine and Ang II. This was intimately related to the increased vascular reactivity to a vasoconstrictor, possibly through the noncalcemic effect of 1,25(OH)₂D₃.

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References


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