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

D　isturbed 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.¹ Recent reports² 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.³⁻⁴ 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,⁵ and 1,25(OH)₂D₃ constricted the renal vasculature in Sprague-Dawley rats.⁶

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,⁷⁻⁹ 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.¹⁰ In addition to its calcemic effect, 1,25(OH)₂D₃ has noncalcemic effects, such as stimulating cell differentiation,¹¹ depressing parathyroid hormone,¹² and enhancing the immune response.¹³ 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

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jugular vein were cannulated as described above. Then, after the pressor response experiment, blood samples were cannulated with PE-50 polyethylene tubing. The procedure was repeated 15 minutes after MAP returned to basal level. Each injection was done 15 minutes after MAP returned to basal level. Each injection was done 15 minutes after MAP returned to basal level.

In twenty-three Sprague-Dawley rats, 1,25(OH)2D3, 1,25-dihydroxyvitamin D3; OCT, 22-oxacalcitriol; 24,25(OH)2D3, 24,25-dihydroxyvitamin D3; BW, body weight; MAP, basal mean arterial pressure; HR, basal heart rate; bpm, beats per minute; s-Ca, serum calcium. Data are mean±SEM.

\*p<0.01 vs. control.

### Table 1. Body Weight, Blood Pressure, and Serum Calcium Level in Experimental Rat Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle (n=14)</th>
<th>1,25(OH)2D3 (n=14)</th>
<th>OCT (n=16)</th>
<th>24,25(OH)2D3 (n=10)</th>
<th>Calcium (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>311±2</td>
<td>290±3</td>
<td>302±2</td>
<td>305±3</td>
<td>322±6</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>108±3</td>
<td>109±2</td>
<td>107±2</td>
<td>108±3</td>
<td>107±1</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>390±14</td>
<td>394±22</td>
<td>388±16</td>
<td>402±17</td>
<td>408±21</td>
</tr>
<tr>
<td>s-Ca (mg/dl)</td>
<td>9.8±0.1</td>
<td>11.0±0.2*</td>
<td>10.1±0.2</td>
<td>10.3±0.1</td>
<td>11.2±0.2*</td>
</tr>
</tbody>
</table>

1,25(OH)2D3, 1,25-dihydroxyvitamin D3; OCT, 22-oxacalcitriol; 24,25(OH)2D3, 24,25-dihydroxyvitamin D3; BW, body weight; MAP, basal mean arterial pressure; HR, basal heart rate; bpm, beats per minute; s-Ca, serum calcium. Data are mean±SEM.

\*p<0.01 vs. control.
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Vitamin D and Vascular Tone

50
40
30
CD
S
20
10

FIGURE 1. Line graph shows mean blood pressure (MAP) response to norepinephrine (10^-8, 10^-7, and 0.5 x 10^-6 mol/kg) in Sprague-Dawley rats maintained on various regimens. ○, 1,25-Dihydroxyvitamin D3 (Vit D3) treatment (n=7); □, 22-oxacalcitriol (OCT) treatment (n=8); △, vehicle (Cont) treatment (n=7); ★, 24,25-dihydroxyvitamin D3 (24,25D3) treatment (n=5); +, calcium infusion (Ca) treatment (n=4). 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.

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 x 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. Corresponding to enhanced pressor response, the decrease in heart rate was greater in the 1,25(OH)2D3 and OCT groups than in the control group [-33±14%, -36±13%, and -65±18% in the 1,25(OH)2D3 group, p<0.05; -32±15%, -40±14%, and -60±18% in the OCT group, p<0.05]. Ang II also elevated MAP in control rats, and the pressor responses to Ang II were significantly higher in rats treated with 1,25(OH)2D3 (p<0.01) and OCT (p<0.01) as compared with rats treated with vehicle but were not affected by 24,25(OH)2D3 (Table 2). According to the changes in MAP, heart rate decreased greatly with infusion of norepinephrine or Ang II in both 1,25(OH)2D3- and OCT-treated rats as compared with vehicle- or 24,25(OH)2D3-treated rats (Table 2).

Protocol 2

Basal MAP was not different between rats infused with saline (108±1 mm Hg) or CaCl2 (107±1 mm Hg) (Table 1). CaCl2-infused rats showed elevated serum calcium levels similar to those of the 1,25(OH)2D3-treated rats (Table 1). Both norepinephrine and Ang II

| Table 2. Change in Mean Arterial Pressure and Heart Rate With Angiotensin II |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Group (n=7)     | Angiotensin II (ng/kg) |                |
|                 | 12.5            | 25              | 50              | p (vs. vehicle) |
| Vehicle (n=7)   |                 |                 |                 |                 |
| MAP (mm Hg)    | 9±1             | 18±2            | 29±4            | <0.01           |
| Heart rate (bpm)| -15±12          | -29±15          | -33±16          |                 |
| 1,25(OH)2D3 (n=7) |                 |                 |                 |                 |
| MAP (mm Hg)    | 7±1             | 14±1            | 25±2            | <0.01           |
| Heart rate (bpm)| -22±13          | -30±14          | -58±16          | <0.05           |
| OCT (n=8)      |                 |                 |                 |                 |
| MAP (mm Hg)    | 9±1             | 18±2            | 29±4            | <0.01           |
| Heart rate (bpm)| -27±16          | -37±20          | -64±22          | <0.05           |
| 24,25(OH)2D3 (n=5) |                 |                 |                 |                 |
| MAP (mm Hg)    | 5±1             | 10±2            | 15±3            | NS              |
| Heart rate (bpm)| -16±14          | -31±13          | -34±14          | NS              |
| Calcium (n=4)  |                 |                 |                 |                 |
| MAP (mm Hg)    | 4±1             | 9±1             | 16±2            | NS              |
| Heart rate (bpm)| -13±15          | -28±16          | -34±16          | NS              |

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.
Protocol 3

Body weight [315±1, 288±5, 305±4, and 303±4 g control, 1,25(OH)D₃, OCT, and 24,25(OH)D₃, 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 µmol showed similar trends to that in protocol 1. Changes of perfusion pressure with norepinephrine at 5, 10, 50, and 100 µmol were significantly greater in both VitD₃- and OCT-treated rats as compared with vehicle- and 24,25D₃-treated rats. **p<0.01 (versus vehicle) by Scheffe’s method.

Discussion

Our first observation that 1,25(OH)₂D₃ enhances pressor responses to both norepinephrine and Ang II is consistent with the previous study by Bukoski et al.1 The most important finding is that a noncalcemic analogue, OCT,11,13,15,16 not only enhanced the pressor response to vasoactive substances but also augmented the incremental responses of perfusion pressure to norepinephrine to the same degree as the administration of 1,25(OH)₂D₃ in the mesenteric artery preparation. These results suggest that 1,25(OH)₂D₃-induced augmentation of pressor and vascular responses to vasoconstrictors may be due to the noncalcemic effect of active vitamin D₃.

The noncalcemic analogue OCT has the same physiological activity as 1,25(OH)₂D₃, lowering bone calcium mobilizing activity18 and suppressing parathyroid hormone synthesis and secretion.13 OCT does not elevate intracellular calcium, although 1,25(OH)₂D₃ increases intracellular and extracellular calcium.17,18 OCT was then used to investigate the noncalcemic effects of 1,25(OH)₂D₃. In fact, treatment with OCT could enhance pressor responses to norepinephrine and Ang II despite no apparent increase in serum calcium concentration. This suggests that 1,25(OH)₂D₃ enhances the pressor effect to vasoconstrictive agents through its noncalcemic effect. In the present study, we did not measure 1,25(OH)₂D₃ level, but the effect of OCT must not be mediated through a secondary increase in 1,25(OH)₂D₃, because OCT has been reported to decrease 1,25(OH)₂D₃ level.19,20 We suggest that the enhancing effect of OCT on pressor action may not be mediated through increasing 1,25(OH)₂D₃.

This hypothesis is also supported by the results of the calcium infusion study. Calcium infusion did not affect pressor responses to norepinephrine and Ang II, although serum calcium concentration was elevated to the same level as in 1,25(OH)₂D₃-treated rats. Some investigators21,22 demonstrated that intravenous infusion of a high dose of calcium could enhance pressor effects by norepinephrine. In humans,23 pressor responses to norepinephrine 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 study with femoral artery rings from deoxycorticosterone acetate–salt and Sprague-Dawley rats revealed that high extracellular ionized calcium (2.5 mM) enhanced vasoconstriction by KCl and norepinephrine compared with low calcium (0.25 mM).22 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.23 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,24 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.25 However, Baks and Hughes26 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 D$_3$ on norepinephrine release was not examined. The results of the present study suggest that increased pressor response to norepinephrine by 1,25(OH)$_2$D$_3$ 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)$_2$D$_3$ 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 nongenomic$^{17}$ or genomic pathways$^{16}$ augmented vascular responsiveness. The possible role of changes in intracellular calcium level, however, is still unknown, and further study is required. Recent studies on vasoactive 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)$_2$D$_3$ receptors on endothelial cells.$^{29}$ Although little is known about the effect of 1,25(OH)$_2$D$_3$ on endothelial function, hypervitaminosis D in rats caused endothelial degeneration.$^{30}$ In contrast, a recent study by Xue et al$^{31}$ indicated that 1,25(OH)$_2$D$_3$ preserved endothelial function in culture conditions. In in vitro conditions, vascular relaxation with acetylcholine is well preserved when active vitamin D$_3$ 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)$_2$D$_3$ on endothelial function still remains to be determined.

Finally, because of the active vitamin D$_3$-induced enhancement of pressor and vasoconstrictor responses to vasoactive substances, we should consider the possible role of active vitamin D$_3$ on vascular hypertrophy. Evidence includes the existence of 1,25(OH)$_2$D$_3$ receptors on vascular smooth muscle cells$^{3-5}$ and the 1,25(OH)$_2$D$_3$ modulation of the growth of vascular smooth muscle cells in vitro$^{32,33}$ and in vivo.$^{30}$ If the administration of 1,25(OH)$_2$D$_3$ and OCT in rats could cause vascular hypertrophy, it may also increase vascular pressor 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)$_2$D$_3$ 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)$_2$D$_3$.

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References


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