Effects of Hydrochlorothiazide and Diltiazem on Reflex Vasoconstriction in Hypertension

PRAMOD K. MOHANTY, JAMES R. SOWERS, AND MARC D. THAMES

SUMMARY The purpose of our study was to determine the effects of treatment with hydrochlorothiazide (n = 10) or diltiazem (n = 8) on reflex humoral, hemodynamic, and vascular responses to graded lower body negative pressure in subjects with mild to moderate hypertension (supine diastolic pressure, 95-114 mm Hg). All subjects received placebo for 2 to 4 weeks followed by either hydrochlorothiazide (25-50 mg b.i.d.) or diltiazem (120-180 mg b.i.d.) to achieve a reduction in supine diastolic pressure of 10 mm Hg or more and a final pressure below 90 mm Hg. Mean arterial pressure, forearm vascular resistance, plasma norepinephrine, and renin responses to graded lower body negative pressure (—10, —20, —40 mm Hg) and head-up tilt were examined before and after 12 weeks of treatment with either drug. Pretreatment basal values of mean arterial pressure (114 ± 2 vs 117 ± 2 mm Hg), forearm vascular resistance (29 ± 3 vs 35 ± 7 units), and plasma renin activity (0.7 ± 0.2 vs 0.6 ± 0.2 ng angiotensin I/ml/hr) were not significantly different between groups. There were no significant differences in basal plasma norepinephrine or in the increases of norepinephrine in response to lower body negative pressure before and after treatment in either group. Forearm vascular resistance responses to lower body negative pressure were virtually abolished in the diltiazem-treated group but not in the hydrochlorothiazide-treated group despite similar levels of mean arterial pressure and basal forearm vascular resistance. Plasma renin activity was significantly higher in the hydrochlorothiazide-treated group after therapy, but the change in renin activity in response to lower body negative pressure was similar before and after therapy in either group. These results suggest that the reflex neurogenic vasoconstriction during lower body negative pressure is inhibited by diltiazem through deactivation of calcium-dependent contractile responses of vascular smooth muscle in the forearm. (Hypertension 10: 35-42, 1987)

KEY WORDS • diltiazem • hydrochlorothiazide • forearm vascular resistance • lower body negative pressure

In the majority of patients with essential hypertension the increase in arterial pressure is the result of augmented systemic vascular resistance while cardiac output and blood viscosity remain normal. The increased vascular resistance may be due to 1) structural changes in vasculature, 2) altered vascular reactivity, or 3) neurogenic vasoconstriction. Many of the agents used in the treatment of hypertension may affect some or all of these factors.

Calcium influx can be inhibited by a number of chemically distinct pharmacological agents, including diltiazem, nifedipine, and verapamil, that antagonize calcium entry during vascular smooth muscle cell activation and thus inhibit the contractile process. Previous studies have suggested that vasoconstriction in patients with essential hypertension is dependent on the influx of calcium into vascular smooth muscle. Since calcium plays a major role in α-adrenergic mediated vasoconstriction, it is reasonable to assume that some calcium antagonists have α-adrenergic blocking activity, and indeed, this has been observed previously.

In contrast with calcium channel blocking agents, the antihypertensive effects of diuretics such as hydrochlorothiazide may be due to chronic sodium deple-
tion resulting in a decrease in responsiveness of the efferent sympathetic nervous system or a modification of the affinity of arterial smooth muscle receptors for angiotensin and norepinephrine. Thus, both thiazide diuretics and calcium channel blockers could alter basal vascular resistance as well as vascular responses to reflex sympathetic activation.

The purpose of our study was to assess the influence of hydrochlorothiazide and diltiazem on reflex humoral, hemodynamic, and regional vascular responses to determine whether these effects play an important role in the therapeutic responses to these agents. We used lower body negative pressure (LBNP) to activate the sympathetic nervous system. At low levels of LBNP (−5 to −20 mm Hg), selective unloading of cardiopulmonary baroreceptors but not arterial baroreceptors leads to increased sympathetic outflow, resulting in forearm vasoconstriction and increased muscle sympathetic activity and plasma norepinephrine levels. At higher levels of suction (>−40 mm Hg), both cardiopulmonary and arterial baroreceptors are unloaded, thus resulting in greater sympathoexcitation.

Our results indicate that only diltiazem blocks reflex vasoconstrictr responses. It does so by interfering with vascular smooth muscle contraction rather than by altering catecholamine release.

Subjects and Methods

Our study population consisted of 18 subjects (17 men, 1 woman) with mild to moderate essential hypertension (supine diastolic blood pressure, 95–110 mm Hg on two consecutive visits during 4 weeks without drug therapy). All subjects were randomized to receive either diltiazem or hydrochlorothiazide for 12 weeks. Demographic characteristics of each group are illustrated in Table 1. Subjects admitted to the study showed no clinical evidence of other major medical illnesses or any major complications of hypertension, such as congestive heart failure or renal insufficiency. The studies were approved by the institutional committee on human investigation, and each subject gave informed consent to participate.

Reflex physiological studies and hormonal measurements were performed during the placebo phase (predrug therapy) and during the 12th week of drug therapy for both drug regimens. All antihypertensive medications were discontinued at least 2 weeks before the beginning of the placebo phase.

Before randomization to either drug, subjects received two capsules and one tablet, all placebo, twice daily for 2 to 4 weeks during which arterial diastolic pressure remained between 95 and 115 mm Hg. Therapy with diltiazem, 120 mg twice daily, was initiated in one group, and the dose was increased after 4 weeks to 360 mg/day if the target pressure (supine diastolic blood pressure below 90 mm Hg and a reduction of 10 mm Hg or more) was not achieved. The second group of subjects initially received hydrochlorothiazide, 50 mg/day, and the dose was increased to 100 mg/day as necessary to achieve the diastolic blood pressure response required of the diltiazem-treated subjects.

Baseline hemodynamic measurements and blood sampling were initiated after each subject had assumed a supine position and a heparin lock had been placed in a forearm vein of the left arm for a minimum of 30 minutes. Subjects were positioned in a LBNP chamber (Medical Instruments, University of Iowa, Iowa City, IA, USA) encasing the body below the iliac crest. This chamber was sealed and connected to an adjustable vacuum source. Graded LBNP of −10, −20, and −40 mm Hg was applied. The pressure was maintained at each level for 5 minutes, with a 10-minute rest between each level. Heart rate was derived from an electrocardiogram, which was recorded continuously, and blood pressure was measured by an automated cuff method (Model 1160; Critikon, Tampa, FL, USA).

Forearm blood flow was measured in each subject's right arm by venous occlusion plethysmography using a mercury-in-Silastic strain gauge plethysmograph (Model EC-3; DE Hokanson, Seattle, WA, USA), as described previously in detail. Forearm vascular resistance (FVR) (expressed as units) was calculated by dividing mean arterial pressure (diastolic plus one third of pulse pressure; in mm Hg) by forearm blood flow (expressed as ml/min/dl of forearm volume). Following LBNP studies, subjects remained supine for 30 minutes, after which pretilt blood sampling was performed. Subjects then were tilted head-up at 80 degrees, and measurements of heart rate, blood pressure, and blood sampling were repeated following 10 minutes of tilt.

Blood samples (6 ml) were obtained in prechilled, heparinized tubes before and during the last minute of each level of LBNP and head-up tilt for measurement of plasma catecholamines and aldosterone and plasma renin activity (PRA). The plasma was separated from the red blood cells and stored at −100°C until assayed. Catecholamines were assayed by high-performance liquid chromatography employing electrochemical detection. Detection in this system is coulometric and is able to detect levels as low as 5 pg/ml for norepinephrine and epinephrine. PRA was measured by radioimmunoassay of angiotensin I after titration to pH 7.4 with phosphate buffer and incubation at 37°C in the presence of angiotensinase inhibitors. Sensitivity of this assay is 0.2 ng angiotensin

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Diltiazem (n = 10)</th>
<th>Hydrochlorothiazide (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)*</td>
<td>49 ± 8</td>
<td>53 ± 10</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>10:0</td>
<td>7:1</td>
</tr>
<tr>
<td>Race (black/white)</td>
<td>6:4</td>
<td>5:3</td>
</tr>
<tr>
<td>Duration of hypertension (yr)*</td>
<td>4 ± 3</td>
<td>5 ± 2</td>
</tr>
</tbody>
</table>

*Values are means ± SD.
Results

Hemodynamic Effects

Table 2 illustrates the effect of treatment with either drug. Both drugs decreased systolic, diastolic, and mean arterial pressure significantly (p < 0.01) and comparably in the supine position. Heart rate tended to be somewhat less following diltiazem therapy, but the change was not significant. Basal FVR decreased significantly (p < 0.01) but comparably in both groups.

Figure 1 illustrates the changes in computed FVR that resulted from graded LBNP before and after treatment. Following diltiazem therapy the increases in FVR were significantly reduced (p < 0.001) at all levels of LBNP. In contrast, treatment with hydrochlorothiazide did not change forearm vasoconstrictor responses to LBNP, although the responses tended to be decreased at the two higher levels of suction (see Figure 1). However, as illustrated in Figure 2, when the percentage change in FVR (change in FVR normalized for baseline FVR) was analyzed, it was found to be significantly higher (p < 0.05) in subjects following hydrochlorothiazide treatment, and the change was striking (p < 0.001) at low level (LBNP, −10 mm Hg) suction, which is known to selectively deactivate cardiopulmonary baroreceptors. In contrast with posthydrochlorothiazide treatment values, the percentage change was not significant. Basal FVR decreased significantly (p < 0.001) at all levels of suction after diltiazem treatment. The levels of suction we used did not change mean arterial pressure or heart rate significantly, but heart rate and mean arterial pressure increased with head-up tilt.

Hormonal Responses

Figure 3 illustrates plasma aldosterone and PRA responses to LBNP in hydrochlorothiazide-treated subjects. Basal levels of PRA as well as levels following LBNP were significantly lower (p < 0.01) following hydrochlorothiazide therapy. Basal aldosterone levels tended to be higher after hydrochlorothiazide treatment, and responses to suction were augmented at all levels of suction (p < 0.05). PRA and aldosterone levels both increased (p < 0.025) following tilt before and after hydrochlorothiazide treatment. Diltiazem treatment did not alter the basal levels of PRA and aldosterone and did not alter the responses of these humoral agents to graded LBNP or tilt (Figure 4).

Before diltiazem therapy, LBNP increased norepinephrine from a basal value of 134 ± 55 pg/ml to 253 ± 45 pg/ml (p < 0.01) at −40 mm Hg of negative pressure. Head-up tilt at 80 degrees also was associated with a significant increase (p < 0.01) in plasma norepinephrine from pretilt supine levels (Figure 5). Diltiazem therapy did not change basal supine norepinephrine levels or responses of plasma norepinephrine to graded LBNP or head-up tilt (see Figure 5).

Plasma norepinephrine values at baseline, with LBNP (−10, −20, −40 mm Hg), and with tilt did not differ significantly before and after treatment with hydrochlorothiazide. Thus, neither diltiazem nor hydrochlorothiazide treatment altered norepinephrine levels at baseline or in response to cardiopulmonary baroreceptor unloading by graded LBNP or head-up tilt.

Discussion

The major finding of our study is that diltiazem, a calcium channel blocking agent, attenuated reflex vasoconstriction. In contrast, hydrochlorothiazide, which produced similar reductions in systolic, diastolic, and mean arterial pressure and basal vascular resistance, had an opposite effect on reflex forearm vasoconstriction.

Vascular responses to vasoconstrictor stimuli are known to be augmented in hypertension. The factors that may contribute to augmented sympathetically mediated forearm vasoconstriction in hypertension include increased norepinephrine release, increased circulating nonadrenergic humoral substances (such as angiotensin II) that can amplify responsiveness of vascular smooth muscle to incoming sympathetic impulses, and structural changes and increased vascular reactivity of the vessel walls of hypertensive subjects. 

The difference in vasoconstrictor responses (as determined by change in FVR) to LBNP between the
hydrochlorothiazide-treated group and the diltiazem-treated group may have been related to somewhat higher pretreatment basal FVR in the hydrochlorothiazide-treated group. When the changes of FVR are normalized for the basal FVR (i.e., change in FVR expressed as a percentage of baseline value), the difference in the effects of the two treatments appear to be even more striking (see Figure 2). Diuretics such as hydrochlorothiazide, which influence the sodium content of the body, may account for the enhanced reflex responsiveness of vascular smooth muscle and provide an explanation for the failure of this agent to decrease forearm vasoconstrictor responses (normalized FVR response).

**Figure 1.** Forearm vascular resistance responses (expressed as units) to lower body negative pressure in 10 diltiazem-treated and eight hydrochlorothiazide-treated subjects before and after treatment. Values are means ± SEM. Asterisk denotes a statistical difference (p < 0.001) between pretreatment and posttreatment responses.

**Figure 2.** Normalized forearm vascular resistance (%FVR) responses to lower body negative pressure in 10 diltiazem-treated and eight hydrochlorothiazide-treated subjects before and after treatment. Values are means ± SEM. Asterisk denotes a statistical difference between pretreatment and posttreatment responses; see Results for details.
TABLE 3. Heart Rate and Mean Arterial Pressure at Baseline, During Lower Body Negative Pressure, and During Head-up Tilt Before and After Treatment with Diltiazem and Hydrochlorothiazide

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Baseline</th>
<th>−10</th>
<th>−20</th>
<th>−40</th>
<th>80-degree tilt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-H</td>
<td>78 ± 2</td>
<td>75 ± 3</td>
<td>72 ± 2</td>
<td>80 ± 4</td>
<td>86 ± 3*</td>
</tr>
<tr>
<td>Pre-D</td>
<td>72 ± 3</td>
<td>72 ± 4</td>
<td>71 ± 3</td>
<td>76 ± 3</td>
<td>80 ± 4*</td>
</tr>
<tr>
<td>Post-H</td>
<td>78 ± 2</td>
<td>76 ± 4</td>
<td>80 ± 2</td>
<td>82 ± 3</td>
<td>98 ± 4*</td>
</tr>
<tr>
<td>Post-D</td>
<td>68 ± 2†</td>
<td>66 ± 3†</td>
<td>66 ± 3†</td>
<td>71 ± 4†</td>
<td>82 ± 2*</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-H</td>
<td>117 ± 2</td>
<td>115 ± 3</td>
<td>114 ± 2</td>
<td>115 ± 4</td>
<td>126 ± 3*</td>
</tr>
<tr>
<td>Pre-D</td>
<td>114 ± 2</td>
<td>114 ± 2</td>
<td>113 ± 1</td>
<td>112 ± 3</td>
<td>124 ± 3*</td>
</tr>
<tr>
<td>Post-H</td>
<td>101 ± 3‡</td>
<td>100 ± 3‡</td>
<td>100 ± 3‡</td>
<td>101 ± 4‡</td>
<td>107 ± 3*</td>
</tr>
<tr>
<td>Post-D</td>
<td>105 ± 1‡</td>
<td>102 ± 2‡</td>
<td>101 ± 3‡</td>
<td>100 ± 3‡</td>
<td>105 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SEM. LBNP = lower body negative pressure; H = hydrochlorothiazide; D = diltiazem. *p < 0.01, compared with baseline values. †p < 0.05, compared with posttreatment H values. ‡p < 0.001, compared with respective pretreatment values.

FIGURE 3. Plasma aldosterone and PRA responses to graded lower body negative pressure and 80-degree head-up tilt in eight hydrochlorothiazide-treated subjects before and after 12 weeks of therapy. Values are means ± SEM. Asterisk denotes a statistical difference between prehydrochlorothiazide and posthydrochlorothiazide treatment response; see Results for details.

We also considered the possibility that preserved or increased vasoconstrictor responses after treatment with hydrochlorothiazide (but not diltiazem) may have been influenced by arterial baroreceptor reflexes. This possibility seems highly unlikely since heart rate and arterial pressure responses to LBNP were not significantly different between hydrochlorothiazide-treated and diltiazem-treated groups. Previous studies have suggested that reflex forearm vasoconstriction in response to LBNP at −10 and −20 mm Hg is largely mediated by cardiopulmonary baroreceptor reflexes, since LBNP at −10 and −20 mm Hg does not change blood pressure or heart rate, and thus these low levels of LBNP presumably do not change the stimulus to the arterial baroreceptors. In contrast, LBNP at −40 mm Hg or higher induces vasoconstriction that may be mediated by the unloading of cardiopulmonary as well as arterial baroreceptors, since these levels of LBNP may result in decreased systolic arterial pressure and increased heart rate. Moreover, Abboud et al. have suggested that arterial baroreceptors play only a minor role in the regulation of forearm vasoconstriction.
Figure 4. PRA and aldosterone responses to lower body negative pressure (LBNP) and 80-degree head-up tilt in 10 diltiazem-treated subjects before and after therapy. Values are means ± SEM. Asterisk denotes a statistical difference (p<0.05) between control values and values during intervention (LBNP and head-up tilt).

During LBNP as opposed to splanchnic vasoconstriction which is determined largely by the arterial baroreceptors.

Surprisingly, LBNP of -20 and -40 mm Hg failed to decrease mean arterial pressure in subjects treated with diltiazem. Mulvihill-Wilson et al.24 have shown that suction at -40 mm Hg reduced cardiac output from 5 to 3.4 L/minute. If there was no reflex vasoconstriction, then this level of suction would have resulted in a decrease in mean arterial pressure. In fact, their data demonstrate that chronic administration of prazosin, an α-adrenergic receptor blocker, markedly attenuated reflex vasoconstriction and resulted in large decreases in arterial pressure during LBNP. In light of these observations, we consider it likely that diltiazem blocks reflex vasoconstriction in different regional circulations to different degrees. Vasoconstriction during LBNP in vascular beds other than the forearm may thus have contributed to the maintenance of mean arterial pressure in subjects treated with diltiazem. Parenthetically, the study of Mulvihill-Wilson et al.24 showed prominent α-blockade of forearm vasoconstriction after prazosin treatment, but they also showed similar blockade of responses of total peripheral resistance. Since we did not measure cardiac output in our subjects, we cannot determine the extent to which diltiazem blocked the systemic vasoconstrictor response to LBNP.

There is one very important difference between our protocol and the one used by Mulvihill-Wilson et al.24 Our subjects were returned to basal conditions between each level of LBNP, and the order of the levels of suction was randomized. In their studies, LBNP was continuous and was increased in increments from -8, -16, -32, and -40 mm Hg without returning to baseline. They do not describe the duration of LBNP at each level, but it was most likely rather lengthy. These differences in protocol also may have contributed to the different blood pressure responses observed in the two studies.

Marked attenuation of forearm vasoconstrictor responses following diltiazem treatment in our study points toward a strong dependency of vasomotor tone on calcium influx. Buhler et al.2 have demonstrated significantly greater increases in forearm blood flow following intra-arterial infusions of verapamil and nicardipine in hypertensive subjects as compared with normotensive subjects. In contrast, the increase in flow in response to nonspecific vasodilation with sodium nitroprusside did not differ between the two groups.3 Thus, in our hypertensive subjects, enhanced calcium-dependent vasoconstriction may have contributed significantly to increased basal vascular resistance, and treatment with calcium channel blocking agents may account for marked attenuation of both basal and reflex-induced vasoconstrictor responses.

We were not able to demonstrate any differences in basal plasma norepinephrine levels or norepinephrine responses to LBNP or tilt before and after treatment with diltiazem or hydrochlorothiazide. Although we did not directly determine forearm norepinephrine release, our data suggest that alterations in norepinephrine release from sympathetic terminals did not play a role in the marked attenuation of vasoconstrictor responses to reflex sympathetic activation following diltiazem treatment.
To determine whether the renin-angiotensin system may have influenced the vascular responses seen, PRA was measured before and after therapy. As expected, basal levels of PRA were significantly increased with hydrochlorothiazide treatment. No significant increase in basal levels of PRA was observed following diltiazem therapy, which is consistent with the observation of Inouye et al. In spite of the differences in the effect of treatment on basal PRA, the changes in PRA in response to LBNP were similar before and after therapy with either diltiazem or hydrochlorothiazide. Thus, it seems unlikely that the renin-angiotensin system plays an important role in the forearm vasoconstrictor responses we observed.

We are aware of only one other study that examined the effects of a calcium entry blocker on reflex vasoconstriction. Ferguson and Dorsey found that a single dose of nifedipine (20 mg) administered sublingually to 14 normal subjects did not alter responses to LBNP. We speculate that they would have observed impaired reflex vasoconstriction had their subjects been treated chronically with nifedipine.

In conclusion, decreased forearm vasoconstrictor responses to LBNP after diltiazem treatment appear to result from deactivation of calcium-dependent contractile responses of vascular smooth muscle. Although the magnitude of the decrease in basal blood pressure and vascular resistance reductions were similar following treatment with either diltiazem or hydrochlorothiazide, reflex neurogenic forearm vasoconstriction was markedly inhibited only by diltiazem. Diltiazem may inhibit neurogenic vasoconstriction in vascular beds other than the forearm to a lesser degree. Hypertensive subjects are continuously subjected to environmental stimuli that may result in sympathoexcitation, vasoconstriction, and further increases in arterial pressure. We found that treatment with diltiazem but not hydrochlorothiazide can prevent such neurogenically mediated vasoconstriction, and we feel that this is an especially important observation based on the following reasoning. Responses of hypertensive subjects to treatment generally are assessed from changes in resting arterial pressure. Both hydrochlorothiazide and diltiazem lower arterial pressure (as observed in our study) and would seem equally efficacious based solely on their effects on resting pressure. However, only the calcium channel blocker prevented sympathetically mediated vasoconstriction. We speculate that this type of agent (in this case, diltiazem) may have greater efficacy in preventing the large increases in arterial pressure that occur in response to environmental stresses and result in sympathoexcitation. Studies to test this hypothesis are in progress.

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