Calcium, the Renin-Aldosterone System, and the Hypotensive Response to Nifedipine

LAWRENCE M. RESNICK, JOHN P. NICHOLSON, AND JOHN H. LARAGH

SUMMARY Ionic, hormonal, and blood pressure responses to a single oral dose of the calcium channel blocker nifedipine were assessed in 25 essential hypertensive subjects. When grouped according to their renin-sodium profile, low renin subjects had a greater hypotensive response to nifedipine (change in diastolic blood pressure $-20.0 \pm 1.4$ vs $-6.4 \pm 1.0\%$; $p<0.005$) than did high renin hypertensive subjects. The initial level of serum ionized calcium predicted the blood pressure response to nifedipine ($r=0.70$, $p<0.001$), as did the initial plasma renin activity ($r=0.65$, $p<0.005$). Nifedipine induced a transient rise in serum ionized calcium (from $2.22 \pm 0.02$ to $2.28 \pm 0.02$ mEq/L; $p<0.01$), while plasma renin activity was consistently elevated compared with initial values at 30 ($p<0.01$), 60 ($p<0.01$), and 120 ($p<0.05$) minutes after drug administration. By comparison, plasma aldosterone levels did not rise and even declined at 30 ($p<0.01$), 60 ($p<0.05$), and 120 ($p<0.05$) minutes after nifedipine. These results suggest that low renin hypertension is more critically dependent on extracellular calcium than are higher renin forms and demonstrate that levels of serum ionized calcium, plasma renin activity, or both may predict the sensitivity of blood pressure to calcium channel blockade. Lastly, calcium may play a pivotal role in vivo in coupling renin stimulation to adrenal aldosterone responses. (Hypertension 10: 254-258, 1987)

KEY WORDS • calcium metabolism • renin activity • calcium channel blockade • hypertension

VARIOUS abnormalities of calcium metabolism have recently been described in both experimental and human forms of hypertension, although the specific manner in which these abnormalities relate to the elevations in pressure remains unknown. We have recently demonstrated consistent relationships between circulating levels of ionized calcium and the concurrent plasma renin activity in human essential hypertension. Low renin essential hypertensive subjects had lower average levels of serum ionized calcium compared with both other hypertensive subjects and normotensive control subjects. We hypothesized that lower serum levels of ionized calcium in these subjects reflected an increase in intracellular calcium levels, and thus an altered steady state distribution of calcium between intracellular and extracellular compartments. If this hypothesis is correct, one might expect an enhanced dependence of blood pressure on the extracellular calcium pool in the low renin hypertensive state. Therefore, blood pressure in these subjects ought to be more sensitive to blockade of intracellular calcium accumulation from extracellular sites. Indeed, calcium channel blocking agents were found to lower pressure preferentially in lower renin forms of experimental as well as human hypertension. We would similarly predict that differing initial levels of extracellular calcium would also affect the hypotensive efficacy of calcium blocking drugs. However, no studies to date have related clinical indices of calcium metabolism to the effects of these agents.

Thus, to study the role of calcium metabolism in hypertension, we have investigated the hypotensive and hormonal responses to a single oral dose of the calcium channel blocking agent nifedipine. The results, reported previously in abstract form, support our working hypothesis and suggest that both serum ionized calcium levels and plasma renin activity can predict the relative hypotensive efficacy of acute cal-

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calcium channel blockade. Moreover, nifedipine administration alters both serum ionized calcium levels and the adrenal aldosterone response to renin stimulation.

Subjects and Methods

Twenty-five subjects were diagnosed as having essential hypertension on the basis of initial screening procedures including blood pressures of greater than 150/95 mm Hg on three separate outpatient visits, normal serum potassium concentration, and the absence of other clinical or chemical evidence of secondary forms of hypertension. All subjects were either untreated or were withdrawn from antihypertensive medications at least 3 weeks before study. On the morning of study, after an overnight fast, subjects arrived at the Cardiovascular Center of the New York Hospital–Cornell Medical Center with a 24-hour urine sample collected from the previous day. Subjects sat quietly for 1 hour, and an indwelling intravenous catheter with a three-way stopcock was placed in the forearm for intermittent blood drawing. Blood pressure was measured and recorded automatically every 2 minutes on an Arteriosonde device (Roche Diagnostics, Nutley, NJ, USA). Each subject then received 10 mg of nifedipine (Procardia) p.o. and remained seated for the next 2 hours. Blood was drawn for mineral and hormonal analysis before and 30, 60, and 120 minutes after nifedipine administration.

Two 30-minute periods of ambulation were also performed, one before the hour-long period of seated equilibrium and a second after 120 minutes of nifedipine administration. At the end of each period of ambulation, blood was drawn for analysis of circulating mineral and hormone levels.

Blood pressure results are reported as the percentage of change in diastolic pressure from basal readings, due to the wider variability both in basal pressures and in the systolic blood pressure response. A stable recorded 20-minute baseline period and a 20-minute period at the time of maximal blood pressure response were used for analysis of blood pressure results. Maximal responses were observed 30 to 60 minutes after nifedipine administration. The 24-hour urine collections were analyzed for sodium, calcium, and magnesium content. Blood samples were analyzed for blood urea nitrogen, creatinine, and levels of total and ionized calcium, magnesium, plasma renin activity, and plasma aldosterone. Urine sodium was analyzed by flame photometry, while urine calcium and magnesium levels and blood levels of urea nitrogen, creatinine, and magnesium was measured by Autoanalyzer techniques (Technicon, Tarrytown, NY, USA).

Serum ionized calcium was measured in serum samples drawn and processed anaerobically, using an ion-specific calcium electrode (Applied Medical Technologies, Palo Alto, CA, USA) by Laboratory Procedures (Upjohn, Kalamazoo, MI, USA). The normal range (95% confidence limits) for the reporting laboratory is 1.85 to 2.50 mEq/L. Plasma renin activity and plasma aldosterone levels were measured by radioimmunoassay procedures. 8

Statistical analysis included, when appropriate, one-way analysis of variance, subsequent modified t tests (Bonferroni), analysis of variance for repeated measures with subsequent modified t tests (Bonferroni, or for nonnormally distributed data, Friedman’s test), and linear regression with Pearson correlation coefficients. All results are expressed as means ± SEM.

Results

Clinical Characteristics

Clinical characteristics of the subjects are displayed in Table 1. Subjects were classified as having low, normal, or high renin hypertension on the basis of their plasma renin activity considered in relation to urinary sodium excretion. 8 We have found that a 24-hour urine collection is adequate to assign a reproducible renin-sodium “status” of low, normal, or high to newly investigated essential hypertensive subjects studied under free-living conditions. Subjects did not differ in age distribution, and low and high renin groups did not differ with respect to sex distribution or basal renal function. All groups had comparable initial seated blood pressures. There was a preponderance of men among normal renin subjects, which may have accounted for their somewhat higher, albeit normal, initial blood urea nitrogen and creatinine values. Of the five black subjects, two had low renin and three had normal renin values. Blood pressure and hormonal responses of these subjects did not differ significantly from those of other low or normal renin subjects. Ten of the 16 previously treated subjects had received diuretic therapy; however, their equal distribution among the different renin subgroups (3 of 7 low renin, 5 of 11 normal renin, and 2 of 7 high renin subjects) suggests that prior diuretic treatment does not explain the different metabolic patterns and blood pressure responses observed.

Analysis of divalent cation metabolism among essential hypertensive subgroups is shown in Table 2. Serum levels of basal, seated ionized calcium and magnesium were significantly different among different renin subgroups. In comparison with high renin subjects (n = 7), low renin hypertensive subjects (n = 7) had lower levels of serum ionized calcium (2.18 ± 0.04 vs 2.29 ± 0.06 mEq/L; p < 0.0166) and higher levels of serum magnesium (1.95 ± 0.06 vs 1.75 ± 0.04 mEq/L; p < 0.0166). Low renin subjects had basal urinary calcium excretion only modestly lower than that of high renin subjects (149 ± 19 vs 240 ± 64 mg/day, p = NS) for equivalent levels of urinary sodium excretion (see Table 2).

Blood Pressure Responses to Nifedipine

The blood pressure responses to a single oral dose of nifedipine are displayed in Figures 1 and 2, as well as in Table 2. The blood pressure effects of nifedipine were stable in some, remaining at lower values for the entire 2-hour period after drug administration. In others, a gradual rise toward baseline values was observed. Return of blood pressure values to baseline levels was not observed during the 2-hour period of
Table 1. Clinical Characteristics of Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>Sex (M/F)</th>
<th>BUN (mg/dl)</th>
<th>Cr (mg/dl)</th>
<th>BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low renin (n = 7)</td>
<td>54 ± 2</td>
<td>4:3</td>
<td>15.3 ± 2.5</td>
<td>0.9 ± 0.09</td>
<td>146 ± 6/95 ± 3</td>
</tr>
<tr>
<td>Normal renin (n = 11)</td>
<td>54 ± 4</td>
<td>9:2</td>
<td>17.6 ± 1.8</td>
<td>1.1 ± 0.07</td>
<td>146 ± 7/98 ± 4</td>
</tr>
<tr>
<td>High renin (n = 7)</td>
<td>52 ± 6</td>
<td>4:3</td>
<td>13.0 ± 1.0</td>
<td>0.9 ± 0.06</td>
<td>141 ± 9/97 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SEM. BUN = blood urea nitrogen; Cr = serum creatinine; BP = blood pressure.

Table 2. Renin Activity, Mineral Metabolism, and Blood Pressure Responses to Nifedipine

<table>
<thead>
<tr>
<th>Group</th>
<th>PRA (ng ANG I/ml/hr)</th>
<th>Ca²⁺ (mEq/L)</th>
<th>Mg (mEq/L)</th>
<th>Na (mEq/day)</th>
<th>Ca (mg/day)</th>
<th>Mg (mEq/day)</th>
<th>ΔDBP (mm Hg)</th>
<th>ΔDBP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low renin</td>
<td>0.51 ± 0.09</td>
<td>2.18 ± 0.04</td>
<td>1.95 ± 0.06</td>
<td>79 ± 11</td>
<td>149 ± 19</td>
<td>12 ± 2</td>
<td>-18.8 ± 4.0</td>
<td>-20.0 ± 1.4</td>
</tr>
<tr>
<td>Normal renin</td>
<td>2.3 ± 0.4</td>
<td>2.24 ± 0.03</td>
<td>1.79 ± 0.03</td>
<td>140 ± 24</td>
<td>220 ± 76</td>
<td>13 ± 1</td>
<td>-17.6 ± 8.4</td>
<td>-17.2 ± 2.0</td>
</tr>
<tr>
<td>High renin</td>
<td>8.5 ± 2.2</td>
<td>2.29 ± 0.06*</td>
<td>1.75 ± 0.04*</td>
<td>113 ± 25</td>
<td>240 ± 64</td>
<td>14 ± 3</td>
<td>-6.3 ± 3.2†</td>
<td>-6.4 ± 1.0†</td>
</tr>
</tbody>
</table>

Values are means ± SEM. PRA = plasma renin activity; ANG I = angiotensin I; ΔDBP = nifedipine-induced change in diastolic blood pressure.

*Significance level = 0.05, †significance level = 0.001, compared with values for low renin group. Significance testing based on t tests adjusted for multiple comparisons using Bonferroni method (p<0.0166 for significance level = 0.05, p<0.0003 for significance level = 0.001).

Figure 1. The blood pressure (BP) dependence of short-term nifedipine administration on initial plasma renin activity (PRA). %ΔDBP = maximal percentage of change in diastolic BP from baseline for an individual subject. A₁ = angiotensin I.

Figure 2. The blood pressure (BP) dependence of short-term nifedipine administration on initial serum ionized calcium. %ΔDBP = maximal percentage of change in diastolic BP from baseline for an individual subject.

observation used in this study. All blood pressures 
were stable before nifedipine administration, 
suggesting that further rest itself did not contribute to the 
blood pressure decline observed with nifedipine 
administration.

Although a hypotensive response was observed in 
all subjects and was weakly related to the height of the 
pretreatment diastolic blood pressure (n = 0.27, p = NS), the maximal blood pressure-lowering effect of nifedipine for a given subject was related both to the initial plasma renin activity and to levels of serum ionized calcium. The percentage of change in diastolic pressure among low renin subjects (−20.0 ± 1.4%), having lower average levels of seated ionized calcium (see Table 2), differed significantly from that observed in high renin subjects (−6.4 ± 1.0%), having higher initial serum ionized calcium levels. Normal renin subjects, having intermediate values of serum ionized calcium, displayed intermediate blood pressure responses to nifedipine administration (see Table 2). Furthermore, for all subjects, continuous relationships were apparent between the percentage of change in diastolic blood pressure and the initial ambulatory plasma renin activity (r = 0.65, p < 0.005; see Figure 1), the initial serum ionized calcium level (r = 0.70, p < 0.001; see Figure 2), and the initial magnesium level (n = −0.60, p < 0.01).

Effects of Nifedipine on Ionized Calcium and the Renin-Aldosterone System

The acute effects of nifedipine, analyzed with subjects in the seated position, on the renin-aldosterone
system as well as on serum ionized calcium are shown in Table 3. As can be seen in Table 3, plasma renin activity rose initially and remained modestly but significantly elevated at 30, 60, and 120 minutes after nifedipine treatment. At the same time, plasma levels of aldosterone did not rise in concert with elevations in plasma renin activity, but rather were modestly but significantly suppressed compared with basal, prenifedipine levels (see Table 3). Serum levels of ionized calcium were significantly elevated 1 hour after nifedipine treatment (2.28 ± 0.02 vs 2.24 ± 0.02 mEq/L; p < 0.01) but returned toward basal values thereafter.

Comparing the effect of 30 minutes of ambulation before and after nifedipine administration (60 minutes before vs 150 minutes after nifedipine) revealed a similar pattern. Renin was stimulated to a greater extent by ambulation in the presence of nifedipine (8.8 ± 2.7 vs 6.0 ± 1.7 ng angiotensin I/ml/hr; p < 0.05), while the plasma aldosterone response to upright posture was not significantly different before as compared with after nifedipine administration (19.0 ± 2.4 vs 18.4 ± 2.9 ng/dl).

Discussion

This study investigates calcium metabolism, the renin-aldosterone system, and blood pressure in essential hypertensive subjects before and after a single oral dose of the calcium channel blocker nifedipine. The results confirm the biochemical heterogeneity of divalent cation distributions between renin subgroups of essential hypertension. They also demonstrate that the sensitivity to the acute hypotensive effect of orally administered nifedipine may be predicted by initial levels of ionized calcium, as well as of plasma renin activity. Thus, a low renin and/or lower serum ionized calcium "profile" is associated with an increased sensitivity to nifedipine in comparison with other hypertensive subjects. Lastly, our observations reveal a dissociation in vivo of circulating aldosterone levels from renin activity in the presence of calcium channel blockade.

In a previous study of essential hypertensive subjects, differences in serum levels of both ionized calcium and magnesium were observed in association with differences in plasma renin activity. The present study supports our initial, larger population study, in again demonstrating that subjects with untreated essential hypertension have altered distributions of basal serum ionized calcium and magnesium values when analyzed with respect to plasma renin activity and normalized for the level of urinary sodium excretion. Low renin hypertensive subjects had lower average serum ionized calcium and higher average magnesium levels than did high renin subjects, who displayed the opposite mineral profile (see Table 2). However, a wide range of ionized calcium values was displayed in this study, especially in samples taken after ambulation (see Figure 2). This finding parallels the wide variation in mean normal values (1.85-2.5 [2 SD] mEq/L) of the reporting laboratory. A much narrower range was observed with subjects in the seated position (see Table 2), and thus the possible contribution of ambulation and its attendant sympathetic activation must also be considered. Other technical sources of variation in the ionized calcium measurement, such as variability in pH, cannot be ruled out in this study. However, the significant differences of ion distribution among different renin subgroups remain apparent and form a possible basis for understanding the differing pharmacological responses to nifedipine observed in this study.

Why do hypertensive subjects with lower plasma renin activity and lower serum ionized calcium levels exhibit an enhanced response to nifedipine? Since calcium channel blockade retards cellular calcium uptake through voltage-operated channels from the extracellular space, it seems reasonable to suggest that, in low renin hypertensive individuals, the excess cytosolic free calcium presumed for all hypertensive persons may be more critically dependent on extracellular calcium entry through these channels. Conversely, in high renin, angiotensin II–mediated hypertension, increases in cytosolic free calcium might depend less on

Table 3. Renin, Aldosterone, and Ionized Calcium Responses to Short-term Oral Administration of Nifedipine

<table>
<thead>
<tr>
<th>Variable</th>
<th>PRA (ng ANG 1/ml/hr)</th>
<th>ΔPRA (%)</th>
<th>PA (ng/dl)</th>
<th>ΔPA (%)</th>
<th>Ca2+ (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 minutes after</td>
<td>3.5 ± 3.5</td>
<td>30 ± 4</td>
<td>13.5 ± 1.9</td>
<td>-24 ± 5</td>
<td>2.22 ± 0.02</td>
</tr>
<tr>
<td>120 minutes after</td>
<td>4.6 ± 1.5*</td>
<td>10.2 ± 1.9*</td>
<td>10.5 ± 1.7†</td>
<td>-22 ± 5</td>
<td>2.28 ± 0.02*</td>
</tr>
<tr>
<td>30 minutes after</td>
<td>4.3 ± 1.1*</td>
<td>10.5 ± 1.7†</td>
<td>10.9 ± 2.6†</td>
<td>-20 ± 11</td>
<td>2.24 ± 0.02</td>
</tr>
<tr>
<td>150 minutes after</td>
<td>4.1 ± 1.1†</td>
<td>10.9 ± 2.6†</td>
<td>9.4 ± 7</td>
<td>-4 ± 7</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Amb 1, 2 = Samples analyzed after 30 minutes of ambulation, 1 hour before or 2 hours after nifedipine administration, respectively. PRA = plasma renin activity; ANG I = angiotensin I; PA = plasma aldosterone.

Significance testing used analysis of variance for repeated measures for calcium, with t tests adjusted for multiple comparison using Bonferroni method and Friedman's test for PRA and PA (nonparametric analysis of variance). Ca2+ F = 6.32 with 2.46 df; p < 0.005; PRA Friedman's test statistic = 19.7 with 3 df; p < 0.005; PA Friedman's test statistic = 12.9 with 3 df; p < 0.005.

*p < 0.05, †p < 0.05, compared with values at Time 0.

*p < 0.005, †p < 0.01, compared with Amb 1 values.

*Significance testing used analysis of variance for repeated measures for calcium, with t tests adjusted for multiple comparison using Bonferroni method and Friedman's test for PRA and PA (nonparametric analysis of variance). Ca2+ F = 6.32 with 2.46 df; p < 0.005; PRA Friedman's test statistic = 19.7 with 3 df; p < 0.005; PA Friedman's test statistic = 12.9 with 3 df; p < 0.005.

*p < 0.005, †p < 0.05, compared with values at Time 0.

*p < 0.05, compared with Amb 1 values.
potential-dependent extracellular calcium uptake and more on angiotensin II-mediated calcium release intracellularly from, for example, sarcoplasmic reticulum.11 Thus, these individuals would not be as sensitive to the blood pressure-lowering effects of extracellular calcium channel blockade. A similar approach would also help explain lower serum ionized calcium levels in the low renin, nifedipine-sensitive subject. Lower serum levels would reflect steady state increases in cellular calcium uptake from the extracellular space and would thus be predictive of an enhanced blood pressure sensitivity to nifedipine regardless of whether subjects were classified according to renin subgroups (see Table 2) or individually (see Figure 2). Interestingly, this hypothesis for low renin hypertension, described in greater detail elsewhere,12 may also hold for magnesium. Although we observed higher serum magnesium values in low renin subjects,2 intracellular free magnesium levels appear to be routinely suppressed in essential hypertension.13 Thus, measurement of serum ionized calcium as well as of plasma renin activity may provide clues to the underlying pathophysiology of hypertension and also predict drug responsiveness to calcium blockers.

Preliminary evidence in the literature is also consistent with these working postulates. Thus, in experimental hypertensive models, calcium channel blockade lowered blood pressure to a much greater extent in the low renin, deoxycorticosterone acetate-saline loaded hypertensive rat than in the more renin-dependent, Goldblatt hypertensive rat.3, 14 Similarly, another recent study reported no significant blood pressure effects of nifedipine in the Goldblatt hypertensive dog.15 Conversely, dietary salt loading in two hypertensive rat strains dramatically increased dihydropyridine receptor densities.16 Furthermore, the clinical antihypertensive effect of another type of calcium channel blocking drug, verapamil, is also enhanced in low renin hypertensive individuals,1 and the longer-term antihypertensive efficacy of nifedipine may also be predicted by initial serum ionized calcium levels.17

Finally, the present study suggests the importance of in vivo calcium transport in mediating the tightly coupled renin-induced stimulation of adrenal aldosterone secretion.18 In vitro studies also suggest a pivotal role for calcium in transducing the adrenal aldosterone response to potassium as well as to angiotensin II-mediated renin stimulation.19 Thus, in the present study, during the 2 hours after nifedipine ingestion, despite significant albeit modest elevations in plasma renin activity, serum levels of aldosterone not only failed to rise concomitantly but actually declined (see Table 3). Although the declining aldosterone values may have also reflected both the normal diurnal decline of aldosterone during morning hours and prolonged rest, it is the failure to respond to elevations in renin activity that seemed biologically significant. Accordingly, calcium channel blockade, at least acutely, seems to dissociate the renin-aldosterone system. This dissociation may persist with longer-term nifedipine therapy,17 an effect that may give calcium channel blocking drugs a particular advantage over other forms of drug therapy (e.g., diuretic or other vasodilating agents) whose effectiveness may be limited by secondary hyperaldosteronism.

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References

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