Enhanced Vasodilatation in Essential Hypertension by Calcium Channel Blockade with Verapamil

U. Lennart Hultén, M.D., Peter Bolli, M.D., Franz W. Amann, M.D., Wolfgang Kiowski, M.D., and Fritz R. Bühler, M.D.

SUMMARY The dependency of arteriolar tone on calcium influx was studied in 11 patients with essential hypertension (EH) and compared to 11 age-matched normotensive subjects (NT) by measuring the forearm blood flow response to intraarterial infusion of the calcium channel blocker verapamil (Verap) and the nonspecific vasodilator sodium nitroprusside (Nip) using venous occlusion plethysmography. Verap in incremental dosages from 1 to 75 μg/100 ml forearm tissue induced a greater increase in forearm blood flow (ΔFAF) in EH than in NT, whereas there was no significant difference in ΔFAF following Nip 1.2 μg/100 ml tissue. ΔFAF to Verap as adjusted for ΔFAF to Nip was still greater in EH than in NT. ΔFAF to all dosages of Verap correlated positively with basal plasma epinephrine concentration in EH. At the two highest dosages of Verap, systemic blood pressure fell in EH, and the Verap-induced vasodilatation (as adjusted for the response to Nip) correlated negatively to plasma renin activity or plasma angiotensin II concentration. These findings support the concept of an increased dependency of arteriolar tone on calcium influx in EH, which is related to the activity of the sympathetic nervous system. This association may be due to a common underlying derangement in transmembranous ionic fluxes in the vascular smooth muscle cells and sympathetic neurons in EH.

(Hypertension 4 (suppl II): II-26-II-31, 1982)

KEY WORDS arteriolar tone • forearm blood flow • essential hypertension • calcium influx • verapamil • sodium nitroprusside • sympathetic activity • renin-angiotensin II.

CYTOPLASMATIC free calcium concentration is the final determinant of the contractile process in the vascular smooth muscle cell. Calcium influx through calcium channels is considered to be a major determinant of free calcium concentration in these cells. Hence, intraarterial infusion of verapamil and nifedipine, both blockers of calcium channels, were shown to produce a marked vasodilatation in man.

An increased dependency on calcium influx for contraction activation as well as a greater relaxation in the presence of verapamil and nifedipine have been demonstrated in blood vessels from spontaneously hypertensive rats as compared to normotensive rats. Most recently, administration of nifedipine was reported to decrease blood pressure in hypertensive but not in normotensive subjects. To investigate the dependency of arteriolar tone on calcium influx in patients with essential hypertension (EH) and normotensive subjects (NT), the verapamil-induced increase in forearm blood flow (FAF) was compared to that following the nonspecific vasodilator, sodium nitroprusside, which is not considered to inhibit calcium influx.

Subjects and Methods

Eleven NT women aged 21–59 years (mean, 49 years) and 11 women with uncomplicated EH (WHO Stage I-II) aged 38–62 years (mean, 49 years) were studied. Antihypertensive treatment was withdrawn at least 1 month prior to the study. All patients had a casual diastolic blood pressure on several occasions ≥ 100 mm Hg (Korotkoff Phase V). The NT were not taking any medication and had no family history of hypertension. Their casual diastolic pressure was ≤ 85 mm Hg. Informed consent was obtained from all individuals.

Forearm Blood Flow (FAF) Measurement

FAF was measured by venous occlusion plethysmography. A mercury silastic strain gauge was placed at the upper third of the forearm, which rested
comfortably on a support slightly above the level of the heart. The strain gauge was coupled to an electronically calibrated plethysmograph (Hokanson EC 3). Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 40 mm Hg by a rapid cuff inflator (Hokanson EC 10). The hand was excluded from the circulation by placing a pediatric blood pressure cuff around the wrist and inflating it to a pressure of 50 mm Hg above the systolic blood pressure for 1 minute prior to and during the measurement of FAF. Determinations of FAF were made by analyzing six (basal blood flow) or four (during maximal vasodilatation) consecutive flow curve recordings, each lasting at least 10 seconds; mean values were taken for statistical evaluation. All measurements were recorded on a Hewlett Packard polygraph.

Study Protocol

The investigations started at 8 a.m. and lasted for about 3 hours. The subjects were supine in a quiet, air-conditioned room with a constant temperature of 20° to 22° C. The forearm volume was measured by water displacement according to the principle of Archimedes. Under local anesthesia a catheter (Autocath, Plastimed, Saint-Leu-La Forêt, France) was inserted into the left brachial artery for regional infusions and for monitoring blood pressure using a Statham P 23 DB pressure transducer. The subjects then rested for 30 minutes, after which arterial blood samples were collected for determination of plasma norepinephrine and epinephrine concentrations, plasma renin activity, and plasma angiotensin II concentration.

Verapamil (Verap) (Isoptin, Knoll AG, Ludwigshafen Federal Republic of Germany), diluted in physiological saline, and sodium nitroprusside (Nip) (Nipride, Hoffmann La Roche AG, Basel, Switzerland) diluted in 5% dextrose solution, were infused into the left brachial artery for regional infusions and for monitoring blood pressure using a Statham P 23 DB pressure transducer. The subjects then rested for 30 minutes, after which arterial blood samples were collected for determination of plasma norepinephrine and epinephrine concentrations, plasma renin activity, and plasma angiotensin II concentration.

Verapamil (Verap) (Isoptin, Knoll AG, Ludwigshafen Federal Republic of Germany), diluted in physiological saline, and sodium nitroprusside (Nip) (Nipride, Hoffmann La Roche AG, Basel, Switzerland) diluted in 5% dextrose solution, were infused into the left brachial artery for constant rate infusion pump (Sage). Verap was given in seven stepwise increase in FAF in both groups (table 2). At the lower dosage levels, FAF had returned to baseline values before infusion of the next dosage whereas semicumulative FAF values were obtained with the higher dosages of Verap. The increase in FAF induced by Verap at all dosages from 1 to 75 µg/100 ml tissue

of 0.6 µg/min/100 ml forearm tissue (0.6 ml/min) for 2 minutes at about 20 minutes after administration of the highest dosage of Verap. FAF was measured from 1 to 4 minutes after starting each infusion. The maximal FAF values were registered between 1 and 2 minutes after Verap and between 2 and 3 minutes after Nip. To assess if the above dosages induced a maximal vasodilatation, intraarterial infusions of Verap 150 µg/min/100 ml tissue for 1 minute and of Nip 1.5 µg/min/100 ml tissue for 2 minutes were additionally given in 13 (7 NT and 6 EH) and nine (6 NT and 3 EH) of the subjects, respectively. FAF was also measured in the right noncatheterized forearm simultaneously with the left throughout the investigation in seven NT and all EH in order to depict spontaneous changes in forearm circulation. Intraarterial blood pressure (BP) was measured before and 3 minutes after the start of each infusion; electrocardiogram (ECG) was monitored throughout the investigation.

Statistical analysis of data was performed on an Apple II desk computer using the Student's t test for paired and unpaired data, the multivariate analysis of variance, as well as linear regression analysis. The values are given as means ± standard error of the mean (SEM), and the level of significance is taken as p < 0.05.

Results

Under basal conditions, intraarterial blood pressure was 125 ± 4 / 64 ± 1 mm Hg in NT and 164 ± 6 / 89 ± 2 mm Hg in EH (p < 0.001 for both). FAF on the left and on the right side as well as heart rate was somewhat but not significantly higher in EH as compared to NT (table 1). Plasma norepinephrine (PNE), epinephrine (PE), plasma renin activity (PRA), and plasma angiotensin II (PAII) did not differ between the groups.

Infusion of Verap 1–75 µg/100 ml tissue induced a stepwise increase in FAF in both groups (table 2). At the lower dosage levels, FAF had returned to baseline values before infusion of the next dosage whereas semicumulative FAF values were obtained with the higher dosages of Verap. The increase in FAF induced by Verap at all dosages from 1 to 75 µg/100 ml tissue

<table>
<thead>
<tr>
<th>Subject</th>
<th>Intravenous blood pressure (mm Hg)</th>
<th>Heart rate (bpm)</th>
<th>Forearm flow (ml/min/100 ml)</th>
<th>PNE (pg/ml)</th>
<th>PE (pg/ml)</th>
<th>PRA (ng/ml/3 hr)</th>
<th>PAII (pg/ml)</th>
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<tr>
<td>systolic</td>
<td>125 ± 4</td>
<td>64 ± 1</td>
<td>61 ± 2</td>
<td>1.8 ± 0.3</td>
<td>1.8 ± 0.2</td>
<td>196 ± 24</td>
<td>61 ± 15</td>
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<td>68 ± 2</td>
<td>(n = 11)</td>
<td>68 ± 2</td>
<td>2.5 ± 0.2</td>
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<td>288 ± 41</td>
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<tr>
<td>systolic</td>
<td>164 ± 6</td>
<td>89 ± 2</td>
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<td>288 ± 41</td>
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<td>75 ± 3</td>
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<td>p</td>
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NT = normotensive; EH = essential hypertensive; ns = not significant. The values are given as means ± SEM.
TABLE 2. Increase in Forearm Blood Flow (Δ FAF, ml/min/100 ml tissue) Following Intraarterial Infusion of Verapamil (from 1 to 75 μg/100 ml tissue) and Sodium Nitroprusside (Nip) (1.2 μg/100 ml tissue) in NT and EH Women

<table>
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<tr>
<th>Subject</th>
<th>Verapamil</th>
<th>Nip 1.2 μg</th>
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<tr>
<td></td>
<td>1 μg</td>
<td>2 μg</td>
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<tr>
<td>NT</td>
<td></td>
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<tr>
<td></td>
<td>0.9 ± 0.3 (n = 8)</td>
<td>2.2 ± 0.4 (n = 10)</td>
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<tr>
<td>EH</td>
<td>2.5 ± 0.6 (n = 11)</td>
<td>6.6 ± 1.5 (n = 11)</td>
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<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
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NT = normotensive; EH = essentially hypertensive. The values are given as means ± SEM.

was significantly greater in EH as compared to NT. The vasodilatation induced by Nip (1.2 μg/100 ml tissue) was somewhat but not significantly higher in EH (table 2). To adjust for the response to Nip, the increase in FAF to all dosages of Verap was divided by the increase in FAF to Nip in each subject. The adjusted vasodilatory response to Verap was greater in EH than in NT (F = 3.5; p = 0.025; fig. 1). The molar ratio of dosages of Verap and Nip having an equal vasodilator effect was 18:1 in NT and 10:1 in EH. In EH as well as in NT, the increase in FAF to Verap 75 μg/100 ml was greater than to Nip 1.2 μg/100 ml (p < 0.001 for both groups).

In EH but not in NT, mean BP was significantly reduced 3 minutes after starting the infusion of Verap, 40 and 75 μg/100 ml, but not after infusion of Nip (fig. 2). In EH and NT, FAF measured on the right side remained practically unchanged at all dosages of Verap as well as Nip. The increase in FAF following Verap 150 μg/100 ml tissue in 13 of the subjects did not significantly differ from that after 75 μg/100 ml tissue (30.0 ± 3.8 and 28.3 ± 3.3 ml/min/100 ml tissue respectively), and Nip 3 μg/100 ml tissue also caused a vasodilatation comparable to that of 1.2 μg/100 ml tissue in the nine individuals studied (25.7 ± 4.7 and 23.1 ± 3.9 ml/min/100 ml tissue respectively). Thus, the vasodilatation induced by Verap 75 μg and Nip 1.2 μg/100 ml tissue can be regarded as maximal.

In NT but not in EH, age correlated negatively with the percentage increase in FAF to Verap 5-75 μg/100 ml (r = -0.641 to -0.880; p < 0.001 to 0.05) and Nip.
1.2 μg/100 ml (r = -0.709; p < 0.05). Basal PE correlated positively to the increase in FAF following all dosages of Verap in EH (r = 0.733 to 0.944; p < 0.001 to 0.05) but no significant correlations were found in NT (fig. 3). Basal PNE correlated positively to the increase in FAF to Verap at the dosages of 1 and 5 μg/100 ml tissue in EH (r = 0.747; p < 0.01 and r = 0.660; p < 0.05, respectively), but no significant correlations were found in NT.

In EH there was a negative correlation between PRA and the vasodilatory response to Verap when expressed as the ratio of increase in FAF to Verap 75 μg/Nip 1.2 μg/100 ml tissue (r = -0.619; p < 0.05) (fig. 4). A negative correlation was also found in EH between basal PAII and the vasodilatory response to Verap expressed as the ratio of increase in FAF to Verap 40 μg/Nip 1.2 μg/100 ml tissue (r = -0.624; p < 0.05).

**Figure 3.** Relationship between basal epinephrine concentration and increase in forearm blood flow (ΔFAF) following infusion of verapamil (Verap) 5 μg/100 ml tissue in nine normotensive women (open circles) and 11 women with essential hypertension (black circles).

**Figure 4.** Left Panel. Relationship between basal plasma renin activity (PRA) and the increase in forearm blood flow (ΔFAF) to verapamil (Verap) 75 μg/100 ml tissue as adjusted for ΔFAF to sodium nitroprusside (Nip) 1.2 μg/100 ml tissue in 11 women with essential hypertension. Right Panel. Relationship between basal plasma angiotensin II concentration and ΔFAF to Verap 40 μg/100 ml tissue as adjusted for ΔFAF to Nip 1.2 μg/100 ml tissue in 11 women with essential hypertension.
Discussion

The calcium channel blocker Verap induced a greater increase in FAF in EH as compared to age-matched NT. The increase in FAF in response to the non-specific vasodilator Nip did not differ in EH and NT groups. When the increase in FAF to Verap was adjusted for the increase in FAF to Nip, it was also greater in EH than in NT. The molar ratio of dosages of Verap and Nip having an equal vasodilator effect was lower in EH (10:1) than in NT (18:1). This ratio in NT was similar to that (16:1) found by Robinson et al.3 Our findings suggest a functional abnormality in essential hypertension with increased dependency of arteriolar tone on calcium influx. This concept is in agreement with observations in the spontaneously hypertensive rat11-12 and with recently presented data indicating an enhanced response to Verap as related to Nip in human essential hypertension.13

The finding that the maximal increase in FAF to Verap was greater than that to Nip in EH as well as in NT suggests that calcium influx is of major importance for the state of tension in the vascular smooth muscle cell. The positive correlations found in EH between basal plasma catecholamines and the increase in FAF to Verap suggest that the vasodilatory response to Verap in EH is augmented with increasing sympathetic activity. This is analogous to the findings that in EH the increase in FAF after alpha-adrenoceptor blockade with prazosin and phentolamine correlates positively to PE and PNE, respectively.3,14 It is well known that increased calcium influx plays a major role for alpha-adrenoceptor-mediated vasoconstriction,4 and alpha-adrenoceptor blocking activity of Verap has been observed both in vivo50 and in vitro.51 However, the maximal vasodilatory response to prazosin and to phentolamine is less than one-third of that induced by Verap, suggesting a more direct influence of catecholamines on calcium channels, and thus on calcium influx. Atlas and Adler52 recently reported data indicating specific binding of the alpha-adrenergic antagonist WB-4101 to calcium channels in rat brain membranes and inhibition of Ca**-mediated depolarization by WB-4101 in neuroblastoma-glioma hybrid cells. Furthermore, the association between the vasodilatory response to Verap and plasma catecholamines in EH may point toward a common underlying derangement in transmembranous ionic fluxes in vascular smooth muscle cells and sympathetic neurons.50,51

The negative correlations between the increase in FAF to Verap as adjusted for the response to Nip and PRA or PAII was only observed at the two highest dosages of Verap, i.e., those that were associated with a fall in systemic blood pressure. Hence, these relations might be due to a compensatory increase in renin release in patients with higher basal PRA8 leading to an attenuation of the Verap-induced vasodilatation. Although measurement of PRA and PAII was not performed in our study after Verapamil titration, an increase in PRA directly related to basal PRA has been reported after acute administration of nifedi-
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Enhanced vasodilatation in essential hypertension by calcium channel blockade with verapamil.
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Hypertension. 1982;4:26-31
doi: 10.1161/01.HYP.4.3_Pt_2.26

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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