Calcium Antagonist Treatment by Lercanidipine Prevents Hyperpolarization in Essential Hypertension

Stefano Taddei, Agostino Virdis, Lorenzo Ghiadoni, Daniele Versari, Guido Salvetti, Armando Magagna, Antonio Salvetti

Abstract—Essential hypertension is associated with impaired endothelium-dependent vasodilation caused by oxidative stress–induced nitric oxide (NO) breakdown and compensatory production of a hyperpolarizing factor. To test whether calcium antagonist treatment can restore NO availability and prevent hyperpolarization through antioxidant properties, in 15 healthy subjects and 15 patients with essential hypertension, we studied forearm blood flow (strain-gauge plethysmography) modifications induced by intrabrachial bradykinin (5, 15, 50 ng/100 mL per minute), an endothelium-dependent vasodilator, in basal conditions, during infusion of N\(^{G}\)-monomethyl-L-arginine (L-NMMA, 100 \(\mu\)g/100 mL per minute), an NO-synthase inhibitor, and ouabain (0.72 \(\mu\)g/100 mL per minute), an Na\(^+\)-K\(^+\) ATPase inhibitor to prevent hyperpolarization. These infusions were repeated in the presence of the antioxidant vitamin C (8 mg/100 mL/min). The response to sodium nitroprusside was also evaluated. In controls, vasodilation to bradykinin was inhibited by L-NMMA and remained unchanged by ouabain or vitamin C. In hypertensive patients, vasodilation to bradykinin was blunted and resistant to L-NMMA but sensitive to ouabain. Vitamin C increased the response to bradykinin and restored the inhibiting effect of L-NMMA while preventing the effect of ouabain. In hypertensive patients, infusions were repeated after 3-month treatment with lercanidipine (10 to 20 mg daily). Lercanidipine decreased plasma lipoperoxides, isoprostanes, and malondialdehyde and increased plasma antioxidant capacity. Moreover, lercanidipine increased the vasodilation to bradykinin and restored the inhibiting effect of L-NMMA on bradykinin-induced vasodilation while preventing the effect of ouabain. Finally, vitamin C no longer exerted its facilitating activity. These results indicate that in essential hypertension, lercanidipine increases endothelium-dependent vasodilation by restoring NO availability and preventing hyperpolarization, an effect probably determined by antioxidant activity. (Hypertension. 2003;41:950-955.)

Key Words: hypertension, essential ■ endothelium ■ nitric oxide ■ endothelium-derived factors ■ free radicals ■ antioxidants ■ calcium antagonists

Endothelium plays a primary role in the modulation of vascular tone\(^1\) by producing and releasing relaxing substances including nitric oxide (NO)\(^2\) and a not-yet identified hyperpolarizing factor (EDHF).\(^3\) In the presence of several cardiovascular risk factors, endothelial cells can also produce contracting factors (EDCFs), which are mainly cyclooxygenase-dependent prostanoids (thromboxane A2 and prostaglandin H2)\(^4,5\) or superoxide anions.\(^6\)

Essential hypertension is characterized by impaired endothelium-dependent vasodilation to specific agonists\(^7-9\) as the result of a reduction in NO oxide availability\(^10,11\) caused by production of oxidative stress.\(^12\) In the presence of impaired NO availability, endothelium-dependent relaxation seems to be sustained by hyperpolarization, which probably acts as a compensatory mechanism.\(^13\)

Since endothelial dysfunction and oxidative stress are promoters of atherosclerosis\(^14-16\) and are closely related to cardiovascular events,\(^17-19\) it is conceivable that an adjunctive target for antihypertensive treatment, in addition to blood pressure lowering, could be represented by restoration of NO production, could be represented by prevention of oxidative stress and restoration of NO availability.\(^20\)

Although several antihypertensive drugs can increase endothelium-dependent vasodilation in patients with essential hypertension, few results are available concerning the mechanism underlying this effect.\(^20\) This aspect is crucial, since the widespread concept that treatment-induced augmented response to an endothelial agonist is an index of increased NO production is highly misleading. Thus, given the above-described possibility of compensatory relaxation mechanisms,\(^13\) when no experimental demonstration is given, in several circumstances the mere increase in agonist-induced vasodilation cannot be extrapolated as an increase in NO availability.
In different vascular districts, calcium antagonists can improve endothelium-dependent vasodilation,\textsuperscript{20} an effect probably related to an antioxidant activity that can lead to the restoration of NO availability.\textsuperscript{21,22} However, it remains to be elucidated whether calcium antagonist–based treatment can prevent production of the hyperpolarizing factor. Thus, in the current study, we evaluated whether in patients with essential hypertension, treatment with the dihydropyridine calcium antagonist lercanidipine\textsuperscript{23} may restore endothelium-dependent vasodilation by improving NO bioavailability and preventing hyperpolarization and whether the mechanism involved could be related to antioxidant activity.

### Methods

#### Patients

The study population included 15 normotensive control subjects and 15 matched patients with essential hypertension (Table). Individuals smoking more than 5 cigarettes per day and/or consuming more than 60 g of ethanol per day were excluded. The protocol was approved by the Ethics Committee of the University of Pisa, and all patients gave their written consent. Hypertensive patients were enrolled if never treated (n=12) or reporting a history of discontinued and ineffective pharmacological antihypertensive treatment (n=3). To avoid possible dropouts, hypertensive patients were tested for response (blood pressure decrease >10%) to a single administration of L-NMMA, 30 minutes' washout was allowed after L-NMMA. and vitamin C were started 10 minutes before bradykinin and continued throughout. Thirty minutes’ washout was allowed between each infusion, whereas a 60-minute period was allowed after L-NMMA.

In hypertensive patients, this experimental design was performed at baseline and after 3-month lercanidipine treatment at 10 mg once daily. After 4 weeks, lercanidipine was titrated to 20 mg once daily in those patients whose blood pressure was found to be higher than 140 to 90 mm Hg (3 of 15). Blood pressure was determined by a mercury sphygmomanometer as the mean of 3 measurements taken at 2-minute intervals after the patients had been seated for 10 minutes.

Finally, at baseline and after 3-month lercanidipine treatment, oxidative stress was evaluated by measuring plasma lipoperoxides with a colorimetric method,\textsuperscript{31} plasma isoprostanes by the ELISA assay after 2 extractions,\textsuperscript{32} and plasma malondialdehyde (MDA) by spectrophotometric assay.\textsuperscript{33} Antioxidant capacity was measured as plasma total antioxidant capability by measuring ferric reducing antioxidant power (FRAP, spectrophotometric assay).\textsuperscript{34}

#### Data Analysis

Results are expressed as mean±SD. Differences between 2 means were compared by the paired Student t test. Responses to bradykinin and sodium nitroprusside were analyzed by ANOVA for repeated measures. Since basal FBF proved to be different in the various experimental steps, data were also analyzed as percent increase or decrease from baseline. Computations for the statistical method were performed with the use of the SAS System.

#### Results

Basal systemic demographic, hemodynamic, and humoral characteristics for normotensive subjects and patients with essential hypertension are summarized in the Table. Age, sex, plasma cholesterol, glycemia, and smoking history were similar and within a normal range between the 2 study groups, who differed in blood pressure (Table).

In patients with essential hypertension, administration of lercanidipine significantly decreased blood pressure values.
after 12 weeks of treatment, whereas heart rate was not modified (Table). Body weight, lipid profile, and glucose plasma levels were unchanged throughout the treatment period (Table).

**Endothelium-Dependent NO Availability, Hyperpolarization, and Oxidative Stress at Baseline**

The FBF increase induced by bradykinin was significantly reduced in patients with essential hypertension (from 2.8±0.5 to a maximum of 12.3±1.8 mL/100 mL per minute; percent increase 343±88.5) as compared with normotensive subjects (from 2.9±0.4 to a maximum of 17.2±2.0 mL/100 mL per minute; percent increase 514±151) (Figure 1). In contrast, vasodilation to sodium nitroprusside was similar in normotensive subjects (from 3.0±0.3 to 13.8±2.0 mL/100 mL per minute; percent increase 357±58) and hypertensive patients (from 2.8±0.5 to 12.6±2.2 mL/100 mL per minute; percent increase 353±85) (Figure 1).

In normotensive subjects, L-NMMA infusion decreased basal FBF (from 2.9±0.3 to 1.9±0.4 mL/100 mL forearm tissue per minute) and significantly (P<0.01) blunted the vasodilator effect of bradykinin (from 1.9±0.4 to 7.0±0.5 mL/100 mL forearm tissue per minute; percent increase, 283±110), whereas ouabain decreased basal FBF (from 3.0±0.2 to 2.3±0.3 mL/100 mL forearm tissue per minute; percent decrease, 25±7) but did not change the response to the agonist (from 2.3±0.0.3 to 13.8±1.8 mL/100 mL forearm tissue per minute; percent increase, 519±65) (Figure 2). In patients with essential hypertension, L-NMMA, which caused a lesser decrease in FBF (from 2.8±0.4 to 2.0±0.3 mL/100 mL forearm tissue per minute) as compared with control subjects (percent decrease, 28% versus 36%, respectively; P<0.01), did not change the response to bradykinin (from 2.0±0.3 to 8.4±1.4 mL/100 mL forearm tissue per minute; percent increase, 338±83) (Figure 2). In contrast, ouabain caused an FBF decrease comparable to L-NMMA (from 2.8±0.5 to 2.0±0.3 mL/100 mL forearm tissue per minute) and significantly (P<0.01) blunted the response to bradykinin (from 2.0±0.3 to 4.9±0.9 mL/100 mL forearm tissue per minute; percent increase, 152±50) (Figure 2).

Finally, in normotensive subjects, vitamin C infusion did not alter basal FBF or change either the response to bradykinin (from 2.9±0.3 to 16.9±2.2 mL/100 mL forearm tissue per minute) or the inhibiting effect of L-NMMA on vasodilation to bradykinin (from 1.9±0.3 to 7.5±1.8 mL/100 mL forearm tissue per minute) (Figure 2). Under vitamin C administration, again ouabain did not alter vasodilation to bradykinin (Figure 2). In contrast, in hypertensive patients, vitamin C increased the response to bradykinin (from 2.8±0.5 to 17.8±2.9 mL/100 mL forearm tissue per minute; percent increase, 553±121; P<0.01 versus bradykinin during saline) (Figure 2). Moreover L-NMMA, when tested under vitamin C administration, blunted the vasodilating effect of

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**Figure 1.** Line graphs show FBF increase above basal induced by intra-arterial bradykinin (left) and sodium nitroprusside (right) in normotensive subjects (n=15) (w) and patients with essential hypertension (n=15) at baseline (*) and after 12-week lercanidipine treatment (10 to 20 mg/d) (●). Data are shown as mean±SD and expressed as percent increase above basal. *Significant difference between response to bradykinin before and after lercanidipine treatment (P<0.05).

**Figure 2.** Bradykinin-induced increase in FBF in the absence (left) and presence (right) of vitamin C (2.4 mg/100 mL forearm tissue per minute) under control conditions (saline at 0.2 mL/min) (○) and in the presence of L-NMMA (100 µg/100 mL forearm tissue per minute) (●) or ouabain (0.72 µg/100 mL forearm tissue per minute) (●) in normotensive subjects (n=15) and patients with essential hypertension (n=15) at baseline (A) and after 12-week treatment with lercanidipine (10 to 20 mg/d) (B). Data are shown as mean±SD and expressed as percent increase above basal. *Significant difference between infusion with and without L-NMMA or ouabain (P<0.05).
bradykinin (from 2.0±0.3 to 6.9±1.5 mL/100 mL forearm tissue per minute; percent increase, 242±81; P<0.01 versus bradykinin in the presence of vitamin C) (Figure 2), whereas ouabain no longer inhibited the response to the agonist (from 2.0±0.3 to 13.0±2.4 mL/100 mL forearm tissue per minute; percent increase, 557±113; NS versus bradykinin in the presence of vitamin C) (Figure 2).

Effect of Lercanidipine on Endothelium-Dependent NO Availability, Hyperpolarization, and Oxidative Stress in Patients With Essential Hypertension

Twelve-week lercanidipine treatment significantly (P<0.01) increased vasodilation to bradykinin (FBF: from 2.8±0.4 to a maximum of 15.9±3.6 mL/100 mL forearm tissue per minute; percent increase, 482±158) (Figure 1) over baseline, whereas the response to sodium nitroprusside (FBF: from 2.8±0.4 to 12.2±2.2 mL/100 mL forearm tissue per minute) (Figure 1) was not changed. Under lercanidipine administration, L-NMMA caused a greater reduction in basal FBF over baseline (FBF from 2.8±0.4 to 1.7±0.3 mL/100 mL forearm tissue per minute; baseline—28% versus lercanidipine—40%; P<0.01) and significantly (P<0.001) blunted the response to bradykinin (FBF: from 1.7±0.3 to a maximum of 5.1±1.8 mL/100 mL forearm tissue per minute; percent increase, 205±81) (Figure 2). In contrast, the increased response to bradykinin was not sensitive to ouabain (FBF: from 1.8±0.3 to a maximum of 10.1±4.0 mL/100 mL forearm tissue per minute; percent increase, 478±298) (Figure 2). Moreover, vitamin C no longer increased the vasodilation to bradykinin (FBF: from 2.8±0.4 to a maximum of 16.0±3.5 mL/100 mL forearm tissue per minute; percent increase, 484±131), nor did it modify the inhibitory effect of L-NMMA (FBF: from 1.7±0.3 to a maximum of 5.3±1.0 mL/100 mL forearm tissue per minute) or ouabain (FBF: from 1.8±0.3 to a maximum of 9.7±2.2 mL/100 mL forearm tissue per minute) on the response to bradykinin (Figure 2).

In both normotensive subjects and hypertensive patients, contralateral FBF underwent no significant change (data not shown).

Regarding the effect of treatment on plasma markers of oxidative stress, lercanidipine treatment significantly lowered plasma lipoperoxides (from 4.9±3.2 to 2.6±2.0 μmol/L; P=0.0003), isoprostanes (from 291.8±161.9 to 218.2±95.8 μmol/L; P=0.03), and MDA (from 8.1±2.9 to 6.1±2.8 μmol/L; P=0.0003), whereas it increased FRAP (from 305.0±31.3 to 435.7±142.9 μmol/L; P=0.005).

Discussion

The current investigation indicates that in patients with essential hypertension, 3-month treatment with the dihydropyridine calcium antagonist lercanidipine increases endothelium-dependent vasodilation to bradykinin by restoring NO availability and inhibiting hyperpolarization. Since lercanidipine prevents the effect of the antioxidant vitamin C and decreases circulating parameters of oxidative stress, it is conceivable that the beneficial effect of this calcium antagonist on endothelial function could be related to antioxidant activity.

In agreement with previous observations,13,35 the response to bradykinin but not to sodium nitroprusside was found to be reduced in patients with essential hypertension as compared with normotensive control subjects. Moreover, whereas L-NMMA inhibited the vasodilating response to bradykinin in normotensive subjects, it was ineffective in patients with essential hypertension.13,35 In contrast, in patients with essential hypertension, the response to bradykinin was resistant to ouabain in normotensive subjects but proved to be sensitive to the Na+K+ ATPase inhibitor.13 Finally, in patients with essential hypertension, intra-arterial administration of vitamin C increased the response to bradykinin, restored the inhibiting activity of L-NMMA, and prevented the inhibition exerted by ouabain.13 It is worth noting that the antioxidant did not change endothelial responses in normotensive subjects. These results confirm the presence of endothelial dysfunction in essential hypertension characterized by the presence of oxidative stress, which impairs NO availability and leads to compensatory production of a hyperpolarizing factor.13 This interpretation is in agreement with experimental evidence indicating that NO, via the production of cyclic GMP, can inhibit an inward depolarizing current and/or activate an outward potassium current, an effect that inhibits hyperpolarization. In the presence of impaired NO availability, smooth muscle cells can become sensitive to EDHF.36

Three-month treatment with lercanidipine increased the vasodilation to bradykinin but not to sodium nitroprusside in patients with essential hypertension. Such a finding is in agreement with experimental data indicating that calcium entry blockers increase endothelial function in various animal vessels37,38 and hypertensive patients.39 In addition, lercanidipine treatment increased the L-NMMA-induced vasoconstrictor effect and restored the ability of L-NMMA to blunt the vasodilation to bradykinin. This is in agreement with previous evidence that calcium antagonists can improve basal and agonist-evoked NO availability.21,22

However, the original finding of the present study is that under lercanidipine treatment, the recovered ability of L-NMMA to inhibit vasodilation to bradykinin is associated with disappearance of the ouabain-induced blunting effect on response to the endothelial agonist. Moreover, after treatment, vitamin C did not alter either the vasodilating response to bradykinin or the activity exerted by L-NMMA and ouabain. Taken together, these findings imply that lercanidipine treatment can restore NO availability while preventing the production of a hyperpolarizing factor. It is, however, important to underline that ouabain is not a specific inhibitor of EDHF activity; rather, it can nonspecifically act on several ion transports.3 Although our findings cannot directly prove that ouabain acts by inhibiting hyperpolarization, on the basis of the experimental literature,3 this would seem to represent the most likely explanation.

The mechanism responsible for lercanidipine-induced improvement in endothelial function is very likely to be an antioxidant effect. It is well documented in several experimental models that calcium antagonists, including lercanidipine, show antioxidant properties.39,40 In agreement with this possibility and with previous evidence in humans,41 in the current study lercanidipine treatment not only prevented the
facilitating effect of vitamin C on vasodilation to bradykinin but also decreased plasma values of lipoperoxides, isoprostanes, and MDA while increasing FRAP. Thus, these findings support the possibility that the beneficial activity of lercanidipine treatment on endothelium-dependent vasodilation is related to antioxidant activity. An additional consideration is that other possible mechanisms do not seem to be operating, at least in these experimental conditions. First, mere blood pressure reduction is a very unlikely explanation. Previous extensive evidence has demonstrated that blood pressure normalization per se is not a therapeutic maneuver sufficient to improve agonist-evoked endothelium-dependent vasodila-
tion.\(^{51−53}\) Moreover, during the 3-month treatment period, the other cardiovascular risk factors that can impair endothelial function, including lipid or glycemic profile,\(^{44}\) showed no change. Finally, the possible contribution of the classic effect of calcium antagonists on voltage-gated L-type calcium channels, which are represented on smooth muscle but not on endothelial cells,\(^{45}\) is partially excluded by the finding that lercanidipine treatment did not change the response to sodium nitroprusside, ruling out any effect on endothelial responses mediated by drug activity on smooth muscle responsiveness. However, it is worth noting that lercanidipine can have an effect on calcium-modulated potassium channels, which can in turn be involved in the vasodilatory properties of EDHF.\(^{46}\) The latter mechanism can explain the degree of vasodilation to bradykinin that remains resistant to L-NMMA after lercanidipine treatment.

**Study Limitations**

The present study is lacking in matched, randomized control groups evaluating the effect of placebo or another antihyper-
tensive drug. Although a placebo arm would have been important to exclude a time effect (or regression to the mean), it is very unlikely that a nonspecific mechanism such as the mere study repetition after 3 months could deeply modify the pathways involved in the vascular responses to bradykinin. Moreover, a control group addressing the effect of blood pressure reduction by an antihypertensive drug would have been useful to prove the specificity of the lercanidipine effect. However, previous evidence is available demonstrating that in hypertensive patients randomly assigned to treatment with lacidipine or atenolol, the \(\beta\)-blocker did not change the vasodilation induced by bradykinin despite a similar blood pressure control.

An additional problem is whether results obtained in the peripheral circulation can be applied to other vascular dis-
tricts. Although no evidence demonstrates a strong correla-
tion between endothelial responses in different vascular beds of the same subjects, it is well documented that endothelial dysfunction is a widespread alteration in the vasculature of patients with essential hypertension. Thus, it is conceivable that the beneficial effect of treatment observed in the periph-
eral microcirculation might be extrapolated to other vascular districts.

**Perspectives**

The present results with lercanidipine further confirm the beneficial effect of calcium antagonists on endothelium-
dependent vasodilation in patients with essential hypertension and provide the demonstration that treatment with this drug can restore an endothelial pathway similar to that observed in normotensive subjects. This is a crucial issue, since the main characteristic of endothelial dysfunction in essential hyper-
tension is production of oxidative stress, leading to impaired NO availability and production of a hyperpolarizing factor as a compensatory pathway.\(^{12−13}\) As NO has important anti-
atherogenic properties,\(^{47}\) whereas endothelial dysfunction and production of oxidative stress, which are characteristic not only of hypertension but also of the most important cardio-
vascular risk factors,\(^{44}\) are associated with cardiovascular events,\(^{17−19}\) it is conceivable that the possibility of restoring NO availability could represent an additional effect of calcium entry blockers, one that would help to prevent the development of atherosclerosis and possibly of clinical events. In line with this interpretation, several clinical studies demonstrate that a calcium antagonist–based treatment can reduce the progression of new atherosclerotic coronary\(^{48}\) or carotid lesions.\(^{49}\) However, the suggestion that restoration of endothelial function by calcium antagonists or by other drug classes active on endothelium-dependent vasodilation could contribute to reduction of cardiovascular events, not only in hypertensive patients but also in subjects with cardiovascular disease, is at the present time only an attractive hypothesis that needs to be demonstrated by specific clinical trials.

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