"Lacidipine prevents endothelial dysfunction in salt-loaded stroke-prone hypertensive rats."

Krenek, Peter ; Salomone, S. ; Kyselovic, J ; Wibo, Maurice ; Morel, Nicole ; Godfraind, Theophile

ABSTRACT

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Lacidipine Prevents Endothelial Dysfunction in Salt-Loaded Stroke-Prone Hypertensive Rats

Peter Krenek, Salvatore Salomone, Jan Kyselovic, Maurice Wibo, Nicole Morel, Théophile Godfraind

Abstract—Endothelium-dependent vasorelaxation is defective in hypertensive rats, especially in conduit arteries. In the stroke-prone spontaneously hypertensive rat, impaired endothelium-dependent vasorelaxation appears to contribute to the pathogenesis of stroke independent of blood pressure. Because treatment with lacidipine, a long-acting calcium channel blocker, protects against stroke and cardiovascular remodeling in this model, we investigated the effect of this treatment on endothelium-dependent vasorelaxation in the aorta. Stroke-prone rats were exposed to a salt-rich diet (1% NaCl in drinking water) with or without lacidipine (1 mg · kg⁻¹ · d⁻¹) for 6 weeks. A high-sodium diet (1) increased systolic blood pressure, aortic weight, and wall thickness and plasma renin activity (P<0.05); (2) markedly reduced nitric oxide (NO)-mediated, endothelium-dependent relaxation of aortic rings to acetylcholine and the sensitivity to the relaxing effect of S-nitroso-N-acetylpenicillamine, an NO donor (P<0.001); and (3) induced an elevation of preproendothelin-1 mRNA levels in aortic tissue (P<0.01) without affecting endothelial NO synthase mRNA levels. Lacidipine treatment prevented the salt-dependent functional and structural alterations of the aorta, including the overexpression of the preproendothelin-1 gene, and increased endothelial NO synthase mRNA levels in aortic tissue (P<0.01). In conclusion, lacidipine protects stroke-prone hypertensive rats against the impairment of endothelium-dependent vasorelaxation evoked by a salt-rich diet, and this effect may contribute to its beneficial effect against end-organ damage and stroke. (Hypertension. 2001;37:1124-1128.)

Key Words: calcium channel blockers ■ calcium antagonists ■ hypertension, sodium-dependent ■ endothelin ■ nitric oxide

The crucial role played by the endothelium in the modulation of vasomotor tone has been increasingly recognized during the past 20 years. Vascular smooth muscle cells respond to various factors produced by the endothelial cells, among which nitric oxide (NO), prostacyclin, and hyperpolarizing factor(s) (EDHF) contribute to relaxation, whereas endothelin-1 (ET-1) and vasoconstrictor prostaglandins promote constriction (see Momboulí and Vanhoutte1 for a review). Impaired endothelium-dependent vascular relaxation has been described in human essential hypertension as well as in animal models of hypertension.1 In the stroke-prone spontaneously hypertensive rat (SHRSP), the endothelial function is deficient, in particular in cerebral arteries,2 and both the severity of endothelial dysfunction3 and the incidence of stroke4 are exacerbated by a salt-rich diet. In this model, impaired endothelium-dependent vasorelaxation appears to contribute to the pathogenesis of stroke independent of blood pressure.5

Calcium channel blockers (CCBs) are widely used in the management of hypertension. Long-term treatment with CCBs has been reported to improve endothelium-dependent vasorelaxation in the salt-sensitive Dahl rat6 and the SHRSP.7 The long-acting CCB lacidipine restores endothelium-dependent vasodilation in patients with essential hypertension.8 In the SHRSP model, lacidipine,9–11 AE004712 and nicardipine10 are able to prevent the occurrence of stroke at dosages that exert hardly any effect on the elevated blood pressure, suggesting that these drugs may have vasculoprotective effects that are unrelated to their antihypertensive effect. Lacidipine treatment of salt-loaded SHRSP, at dosages that had only moderate effects on high blood pressure, indeed counteracted the development of vascular lesions in brain and kidney9 and of vascular remodeling in the basilar13 and mesenteric13 arteries. We have previously reported that lacidipine treatment attenuates myocardial hypertrophy in the SHRSP exposed to a high-salt diet14,15 and in an acute model of pressure overload16 while preventing the salt-related myocardial overexpression of the ET-1 gene. Therefore, we decided to investigate, in the salt-loaded SHRSP model, the effect of long-term treatment with lacidipine on endothelium-dependent vasodilation, vascular structure, and endothelial NO synthase (eNOS) and ET-1 gene expression in the aorta.

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Biometric and Hemodynamic Data and Plasma Renin Activity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SP H2O</th>
<th>SP NaCl</th>
<th>SP H2O Lac</th>
<th>SP NaCl Lac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>255±6</td>
<td>240±9</td>
<td>250±5</td>
<td>254±6</td>
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<td>Systolic blood pressure, mm Hg</td>
<td>220±8</td>
<td>249±6*</td>
<td>190±5*</td>
<td>211±4†</td>
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<tr>
<td>Heart/body wt, mg · g⁻¹</td>
<td>3.46±0.05</td>
<td>3.97±0.11*</td>
<td>3.28±0.03*</td>
<td>3.49±0.03‡</td>
</tr>
<tr>
<td>Aorta weight, mg · mm⁻¹</td>
<td>0.88±0.03</td>
<td>1.02±0.04*</td>
<td>0.83±0.02</td>
<td>0.81±0.03‡</td>
</tr>
<tr>
<td>Aortic wall thickness, μm</td>
<td>90.0±1.5</td>
<td>97.0±2.1*</td>
<td>ND</td>
<td>82.9±2.1†</td>
</tr>
<tr>
<td>PRA, ng · mL⁻¹ · h⁻¹</td>
<td>5.3±0.8</td>
<td>13.0±2.8‡</td>
<td>ND</td>
<td>4.2±0.6†</td>
</tr>
</tbody>
</table>

SP H2O indicates control SHRS; SP NaCl, salt-loaded SHRS; SP H2O Lac, SHRS treated with lacidipine (1 mg · kg⁻¹ · d⁻¹); SP NaCl Lac, salt-loaded SHRS treated with lacidipine (1 mg · kg⁻¹ · d⁻¹); ND, not determined.

*P<0.05 vs SP H2O; †P<0.05 vs SP NaCl; ‡P<0.01 vs SP NaCl.

Methods

Experimental Animals and Tissue Collection
All procedures followed were in accordance with institutional guidelines. Male 8-week old SHRS (Iffa Credo, L’arbresle, France) were divided in random order into 4 groups. Control rats were maintained on ordinary chow and received either salt-free water (SP H2O, n=7) or water containing 1% NaCl (SP NaCl, n=8) as drinking solution. Two groups treated with lacidipine, included in food for a mean daily intake of 1 mg · kg⁻¹, received either salt-free water (SP H2O Lac, n=7) or water containing 1% NaCl (SP NaCl Lac, n=8). Rats were treated for 6 weeks. Systolic blood pressure was measured by tail-cuff method in conscious animals prewarmed to 35°C in thermostatic cages (Physiograph Narcor). At the age of 14 weeks, rats were killed by decapitation, and blood was collected. Plasma was stored at −20°C for later analysis of plasma renin activity (PRA) according to a standard procedure (Medix Biochemica). The aorta (thoracic aorta and arch) was carefully dissected and cleaned of connective tissue in cold (4°C) physiological solution. Two rings (2 mm long) were cut from thoracic aorta and were used to assess vascular reactivity; the other part of the vessel was immediately frozen in liquid nitrogen and stored at −80°C until RNA extraction.

Functional Studies on Isolated Aorta and Measurement of Aortic Hypertrophy
Aortic rings were suspended in organ baths containing a modified Krebs buffer (37°C) composed of (mmol/L): NaCl 122, KCl 5.9, NaHCO3 15, MgCl₂ 1.25, CaCl₂ 1.25, and glucose 11, pH 7.4, and bubbled with a mixture of 95% O₂/5% CO₂. Rings were connected to an isometric force transducer (UC-2 Gould), and resting tension was set to 20 mN. Indomethacin (10⁻⁵ mol/L) was included in all solutions to avoid prostaglandin-mediated effects. Concentration-response curves to acetylcholine (10⁻⁹ to 3×10⁻⁶ mol/L) were performed in preparations precontracted with 10⁻⁶ mol/L norepinephrine. Responses to the NO donor S-nitroso-N-acetylpenicillamine (SNAP, 10⁻⁷ to 3×10⁻⁵ mol/L) were assessed in preparations precontracted with norepinephrine (5×10⁻⁷ mol/L) in the presence of the NO synthase inhibitor L-NAME, 3×10⁻⁴ mol/L, which was added 30 minutes before norepinephrine. Data were collected with a MacLab system and analyzed with the Chart 3.1 software.

After completion of functional studies, rings of thoracic aorta were weighed and fixed in 10% formaldehyde. Their length was measured under a dissection microscope (relative tissue wet weight was expressed as mg · mm⁻¹). Tissue was then paraffin-embedded, and sections 8 μm thick were prepared. Sections were stained by a standard hematoxylin-eosin procedure. Total aortic wall thickness was measured at ×200 magnification. Thickness was measured at 3 equidistant positions along the ring circumference in 5 sections per animal.

RNA Extraction and Northern Analysis
Total RNA from individual aortas was extracted by TriPure isolation reagent (Roche) and stored at −80°C. The average yield of RNA was 20.3±1.2 μg per aorta. Total RNA (15 μg) was subjected to Northern blot analysis essentially as described previously. Membranes were hybridized sequentially with 32P-labeled cDNA probes for preproET-1, eNOS, and GAPDH. Optical densities of preproET-1 and eNOS bands on autoradiograms were expressed relative to GAPDH. Ratios were normalized with respect to RNA samples from untreated rats (SP H2O), which were processed simultaneously.

Drugs
Acetylcholine, norepinephrine, indomethacin, L-NAME, and SNAP were from Sigma. Lacidipine was provided by Glaxo-Wellcome.

Statistical Analysis
Data are reported as mean±SEM. Sensitivity to relaxant drugs was expressed as the negative logarithm of the concentration (mol/L) that caused half-maximal relaxation (pD₂). Comparisons between treatments were made by 1-way ANOVA. The Bonferroni test was used to compare selected pairs of treatments. Probability values <0.05 were considered significant.

Results

Systolic Blood Pressure, Vascular Structure, and PRA
As shown in the Table, systolic blood pressure at 14 weeks of age was increased slightly by prolonged salt loading (P<0.05). Lacidipine (1 mg · kg⁻¹ · d⁻¹) prevented the salt-induced increase in systolic blood pressure (P<0.01) and reduced the blood pressure of SHRS that were not salt-loaded. Aorta weight per unit vessel length and aortic wall thickness were significantly increased by salt (P<0.05). Lacidipine prevented this salt-related vascular hypertrophy (P<0.05) as well as the concomitant cardiac hypertrophy. Confirming earlier results of Volpe et al, we observed that PRA was increased in SHRS exposed to high-salt diet. This PRA increase was prevented by lacidipine treatment (P<0.05). We had found in a previous series of experiments (data not shown) that this lacidipine dosage did not modify PRA in the absence of salt loading.

Vascular Function

Relaxation to Acetylcholine in Norepinephrine-Precontracted Aorta
As shown in Figure 1, contractions to norepinephrine (10⁻⁶ mol/L) were not significantly different between the various groups examined. In aortas isolated from rats exposed to a high-salt diet, the maximum relaxation to acetylcholine was reduced by 50±7.6% (P<0.001), but the sensitivity to
Acetylcholine was hardly changed (pD<sub>2</sub> 7.34±0.06 [n=10] versus 7.22±0.06 [n=8], for control and salt-loaded, respectively). Treatment with lacidipine prevented the salt-related reduction of acetylcholine-induced relaxation but was without detectable effect on the relaxation of aortas from rats not exposed to high salt. The relaxant responses to acetylcholine were suppressed when the preparations were incubated with L-NNA (300 μmol/L), a blocker of NO synthase, indicating that they were related to endogenous NO (data not shown).

Relaxation to SNAP

We used SNAP in the presence of 300 μmol/L L-NNA to analyze the vasorelaxant response to exogenous NO (Figure 2). SNAP was able to completely relax norepinephrine-contracted aortas in each group of SHRSP. However, aortas from salt-loaded rats were less sensitive than control aortas to the relaxing action of SNAP, as demonstrated by the shift to the right of the concentration-relaxation curve (pD<sub>2</sub>, 6.96±0.12 [n=10] and 6.30±0.06 [n=8], for control and salt-loaded, respectively, P<0.001). Treatment of salt-loaded rats with lacidipine largely prevented the decrease in sensitivity to SNAP (pD<sub>2</sub>, 6.71±0.13 [n=8], P<0.05 versus salt-loaded without lacidipine). Lacidipine had no significant effect on SNAP-induced relaxation of aortas from rats on normal salt diet (pD<sub>2</sub>, 6.84±0.15 [n=9]).

PreproET-1 and eNOS Gene Expression in Aortic Wall

Figures 3 and 4 illustrate the results of gene expression analysis. The relative abundance of eNOS mRNA in the vessel wall was not significantly changed after salt loading but was increased by lacidipine treatment in both control and salt-loaded rats (P<0.01). In contrast, preproendothelin-1 mRNA level was elevated 2-fold in the aortas of salt-loaded rats (P<0.01), and this salt-related increase was completely prevented by lacidipine treatment.

Discussion

This study confirms that a salt-rich diet augments cardiovascular hypertrophy in SHRSP<sup>3,13–15</sup> while inducing a paradoxical elevation in PRA, which could be a consequence of renal ischemia<sup>18</sup>. Systolic blood pressure was somewhat increased by salt in this series of SHRSP, whereas no significant difference had been found in previous studies from this laboratory<sup>3,13,14</sup>. High-salt treatment reduced maximal endothelium-dependent relaxation to acetylcholine of the norepinephrine-stimulated aorta and depressed the sensitivity to the relaxing effect of the NO donor SNAP. Acetylcholine-
induced relaxation of the aorta was entirely mediated by NO, as shown by the blocking effect of NO synthase inhibitors. However, eNOS gene expression was not altered, suggesting that the capacity to produce NO was not impaired in the aortic wall by the salt-rich diet. The depressed responsiveness to both acetylcholine and SNAP could be related to an excessive production of reactive oxygen species, which are able to scavenge NO. Increased superoxide anion production by the arterial wall has been reported in several hypertensive models and appears to be responsible for the decrease in NO bioavailability in SHRSP compared with Wistar-Kyoto rats. We cannot exclude, however, that impaired vasorelaxation in salt-loaded SHRSP could be partly due to a reduction in the smooth muscle reactivity to NO, which, according to Barton et al., could be related to the enhanced exposure of smooth muscle cells to ET-1.

We had previously shown that in the SHRSP mesenteric artery contracted by norepinephrine, salt loading impairs endothelium-dependent relaxation to acetylcholine but, unlike in the aorta, increases the responsiveness to SNAP. As discussed by Ghisdal et al., EDHF is an important mediator of the endothelium-dependent relaxation in the mesenteric artery, but its relative contribution decreases in salt-loaded SHRSP, which might account for the greater sensitivity to exogenous NO in this case. Reduced vasorelaxation to acetylcholine has also been reported in salt-sensitive Dahl rats fed a high-salt diet; in this model, the responsiveness to NO donor sodium nitroprusside was reduced (mesenteric artery) or unchanged (aorta), confirming that the effect of exogenous NO differs along the arterial tree and according to the hypertensive model.

Lacidipine treatment prevented vascular functional and structural alterations induced by the high-salt diet in SHRSP while maintaining the blood pressure to a level comparable to that of control SHRSP. It is likely that the cardiovascular and renal (PRA) effects of lacidipine follow, at least partly, from its antihypertensive effect, which was moderate but indisputable at the dosage of 1 mg · kg⁻¹ · d⁻¹. However, blood-pressure-independent protective properties have been postulated to account for the prevention of stroke and myocardial remodeling by this 1,4-dihydropyridine in salt-loaded SHRSP. The effect of lacidipine on eNOS mRNA level is consistent with reports showing that dihydropyridine CCBs stimulate NO production and eNOS expression in cultured endothelial cells. The functional significance of the moderate increase in eNOS gene expression in our model is doubtful because lacidipine treatment had no effect on vasorelaxation to acetylcholine and SNAP in SHRSP that had not been treated with high salt. Alternatively, lacidipine, a potent antioxidant dihydropyridine, could scavenge or block the effects of reactive oxygen species produced in excess in the aortic wall. In agreement with this view, the formation of oxidation-specific epitopes is decreased in arteries of SHRSP exposed to lacidipine. Such an antioxidant action, by increasing the bioavailability of NO, could contribute to reduce vascular ET-1 overexpression. The decrease in aortic ET-1 expression might be related also to the protection by lacidipine against the renal ischemic alterations leading to PRA elevation. Indeed, prevention of PRA elevation by lacidipine would suppress excessive angiotensin II production in the aorta by renin of kidney origin, thereby opposing angiotensin II–stimulated ET-1 gene overexpression in aortic cells. In preliminary experiments on isolated rat aorta, we have observed that in vitro, angiotensin II increases the abundance of preproET-1 mRNA in the aortic wall and that this stimulation is blunted in vessels that had been pretreated with lacidipine. Thus, lacidipine could act not only through renal protection but also by interfering directly with the pathways activated by angiotensin II in the vessel wall. Experiments are in progress to examine this hypothesis. Whichever its mechanism of action, prevention of vascular ET-1 overexpression by lacidipine could maintain the relaxant response to acetylcholine and SNAP by preserving vascular reactivity to NO and also contribute to the antihypertrophic effect of the drug in salt-loaded SHRSP.

Conclusions

The long-acting CCB lacidipine protects SHRSP against the impairment of endothelium-dependent vasorelaxation evoked by a salt-rich diet, and this may contribute to its beneficial effect against end-organ damage and stroke.

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