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

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

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 do...

CITE THIS VERSION
Krenek, Peter ; Salomone, S. ; Kyselovic, J ; Wibo, Maurice ; Morel, Nicole ; et. al. Lacidipine prevents endothelial dysfunction in salt-loaded stroke-prone hypertensive rats.. In: Hypertension, Vol. 37, no. 4, p. 1124-1128 (2001) http://hdl.handle.net/2078.1/8807 -- DOI : 10.1161/01.HYP.37.4.1124

DIAL is an institutional repository for the deposit and dissemination of scientific documents from UCLouvain members. Usage of this document for profit or commercial purposes is strictly prohibited. User agrees to respect copyright about this document, mainly text integrity and source mention. Full content of copyright policy is available at Copyright policy

Available at: http://hdl.handle.net/2078.1/8807
Lacidipine Prevents Endothelial Dysfunction in Salt-Loaded Stroke-Prone Hypertensive Rats
Peter Krenek, Salvatore Salomone, Jan Kyselovic, Maurice Wibo, Nicole Morel and Théophile Godfraind

Hypertension. 2001;37:1124-1128
doi: 10.1161/01.HYP.37.4.1124

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/37/4/1124

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/
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 Mombouli 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, 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 rats⁶ 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 mesenteric 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.
**Methods**

**Experimental Animals and Tissue Collection**

All procedures followed were in accordance with institutional guidelines. Male 8-week old SHRSP (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 · d⁻¹; SP NaCl Lac, salt-loaded SHRSP treated with lacidipine (1 mg · kg⁻¹ · d⁻¹); ND, not determined.

**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 × 10⁻⁴ mol/L) were performed in preparations preconstricted with 10⁻⁶ mol/L norepinephrine. Responses to the NO donor S-nitroso-N-acetylpenicillamine (SNAP, 10⁻⁴ to 3 × 10⁻⁴ mol/L) were assessed in preparations preconstricted with norepinephrine (5 × 10⁻⁷ mol/L) in the presence of the NO synthase inhibitor Nω-nitro-L-arginine (L-NNA, 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 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, GAPDH, and PRA. 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-NNA, 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 SHRSP 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 SHRSP 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 (pD2 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 (pD2 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 (pD2 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 (pD2 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 SHRSP1,13–15 while inducing a paradoxical elevation in PRA, which could be a consequence of renal ischemia.18 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.3,13,14 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-

---

**Figure 1.** Contraction of isolated SHRSP aorta to norepinephrine (A) and concentration-relaxation curves to acetylcholine (B). Aortic rings were precontracted by norepinephrine (10^−6 mol/L) in presence of indomethacin (10^−5 mol/L). When stable plateau was reached, relaxation to acetylcholine (10^−9 to 3×10^−6 mol/L) was obtained. Relaxation is expressed as relative decrease in tone developed after stimulation with norepinephrine (in percent). Data are mean±SEM of 8 to 10 determinations (**P<0.01, ***P<0.001 vs SP H2O). SP H2O indicates control SHRSP; SP NaCl, salt-loaded SHRSP; SP H2O Lac, SHRSP treated with lacidipine (1 mg · kg⁻¹ · d⁻¹); and SP NaCl Lac, salt-loaded SHRSP treated with lacidipine (1 mg · kg⁻¹ · d⁻¹).

**Figure 2.** Contraction of isolated SHRSP aorta to norepinephrine (A) and concentration-relaxation curves to SNAP (B). Same protocol as in Figure 1, except that preparations were precontracted with 5×10^−7 mol/L norepinephrine in presence of L-NNA (300 μmol/L). Data are mean±SEM of 8 to 10 determinations (**P<0.01, ***P<0.001 vs SP H2O).

**Figure 3.** Northern analysis of mRNA levels in aortic tissue: Typical autoradiograms. Blots were sequentially hybridized with 32P-labeled probes for preproET-1, eNOS, and GAPDH mRNA, as described in Methods. NaCl refers to salt given (+) or not given (−) in drinking water (see Methods).
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.

Acknowledgments
This work was supported by grants from the Communauté française de Belgique (Action de recherche concertée no 96/01-199), the Belgian Fonds National de la Recherche Scientifique (F.R.S.M. no 3.4585.00), and Glaxo-Wellcome. We thank Margareta Vandenberg, Marie-Christine Hamaine, and Dr Vaclav Vaja for skillful assistance.

References


