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Enalapril and quinapril improve endothelial vasodilator function and aortic eNOS gene expression in L-NAME-treated rats

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Abstract

Endothelial dysfunction ensuing inhibition of nitric oxide synthase (NOS) was investigated in male Sprague–Dawley rats given N^{ω}-nitro-L-arginine methyl ester (L-NAME) in drinking water for 8 weeks. Age-matched rats served as controls. L-NAME-treated rats, as compared to control animals, showed: (1) a clear-cut increase in systolic blood pressure; (2) a consistent decrease of endothelial-cell NOS (eNOS) gene expression in aortic tissue; (3) a reduction of the relaxant activity of acetylcholine (ACh, from 10^{-10} to 10^{-4} M) on norepinephrine-precontracted aortic rings (reduction by 52 ± 5%); (4) a marked decrease (−50%) of the basal release of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) from aortic rings. In L-NAME-treated rats, administration in the last 2 weeks of either the angiotensin-converting enzyme inhibitor enalapril (1 mg/kg/day) or the cognate drug quinapril (1 mg/kg/day) decreased systolic blood pressure levels, completely restored eNOS mRNA levels in aortic tissue, and allowed a consistent recovery of both the relaxant activity of ACh and the generation of 6-keto-$PGF_{1\alpha}$. No difference was present in the ability of the two angiotensin-converting enzyme inhibitors to reverse NAME-induced endothelial dysfunction. These findings indicate that L-NAME-induced hypertension in the rat relies on the marked impairment of the endothelial vasodilator function, with an ensuing contribution by a decreased production of prostacyclin by the endothelial cells. Angiotensin-converting enzyme inhibition by enalapril or quinapril was equally effective in improving endothelial vasodilator function, prostacyclin endothelial production and restoring aortic eNOS mRNA.

Keywords: Endothelial dysfunction; NO (Nitric oxide); Hypertension; Angiotensin-converting enzyme inhibitor

1. Introduction

Nitric oxide (NO) produced by the vascular endothelium sustains the vasodilator tone and inhibits platelet function, leukocyte adhesion and smooth muscle cell proliferation (Moncada et al., 1991; Radomski et al., 1987; Clozel et al., 1991; Garg and Hassid, 1989). In keeping with these actions, diseases processes such as hypertension, hypercholesterolemia and atherosclerosis share an endothelial dysfunction with abnormal synthesis and/or release of NO (Egashira et al., 1993; Zeiher et al., 1993; Drexler, 1997).

Preclinically, in rats, N^{ω}-nitro-L-arginine methyl ester (L-NAME)-induced blockade of NO synthesis provides a model of specific vascular dysfunction responsible for systemic arterial hypertension and structural alterations of the heart and the arterial walls (Ribeiro et al., 1992; Numaguchi et al., 1995; Takemoto et al., 1997). This complex model of hypertension would also be suitable to test the vascular protective effect of drugs improving endothelial vasodilation function, such as angiotensin-converting enzyme inhibitors. Based on these premises, to get further insight into the endothelial dysfunction induced by NO synthase (NOS) inhibition, we devised first to study in the thoracic aorta from L-NAME-treated rats: (1) the gene expression of endothelial-cell NOS (eNOS) in the vascular tissue; (2) the vasodilator responses of the aortic tissue to acetylcholine (ACh); and (3) the prostacyclin-releasing activity of the aortic tissue. The ability of angiotensin-converting enzyme inhibitors to reverse endothelial dysfunction was then studied by treating rats made hypertensive

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by chronic L-NAME with either enalapril or quinapril. Enalapril is the prototype of the long-acting prodrug ACE inhibitors containing the carboxyl moiety and one of the most widely used angiotensin-converting enzyme inhibitor (Todd and Goa, 1992; Todd and Heel, 1996). Quinapril, a more recently introduced angiotensin-converting enzyme inhibitor also sharing the carboxyl moiety, is a more potent inhibitor than enalapril of tissue angiotensin-converting enzyme, especially in the aorta (Nakajima et al., 1992).

2. Materials and methods

2.1. Animals and treatments

Adult male Sprague–Dawley rats (200–250 g body weight) were purchased from Charles River Italia (Calco, Como, Italy) and were housed under controlled conditions (22 ± 2 °C, 65% humidity and artificial light from 0600 to 2000 h).

Four groups of 10 rats each were studied. In three of the groups, hypertension was induced by administration of the NOS inhibitor L-NAME (Sigma, St. Louis, MO, USA; 60 mg/kg body wt./day) in drinking water for 8 weeks. The fourth group received normal tap water throughout all the experiment (control group). Animals made hypertensive were then treated with either of the following angiotensin-converting enzyme inhibitors given in tap water with L-NAME during the last 2 weeks of treatment: enalapril (1 mg/kg/day) (L-NAME + enalapril), quinapril (1 mg/kg/day) (L-NAME + quinapril). Enalapril and quinapril were used at an almost equipotent hypotensive dose (Todd and Heel, 1996). The third group of hypertensive rats was given only L-NAME with vehicle (L-NAME). Basally and every 2 weeks during the 8 weeks of follow-up, systolic blood pressure was estimated by the tail-cuff method at the same time of day (between 1300 and 1500 h). At the end of the 2-week period of drug administration, all rats were killed by cervical dislocation. From each animal, a segment of the thoracic aorta was rapidly removed for determination of eNOS mRNA levels and evaluation of the endothelial vasodilator function.

The experimental protocol was approved by the Review Committee of the Department of Pharmacology and met the Italian guidelines for use of laboratory animals, which conform with the European Communities Directive of November 1986 (86/609/EEC).

2.2. Reverse transcription-polymerase chain reaction (RT-PCR): aortic eNOS mRNA

Total RNA was isolated from aortic tissues by the single-step acid guanidium–phenol–chloroform extraction (Chomczynski and Sacchi, 1987). RT-PCR was prepared by standard methods with 1 μg of total RNA. First-strand cDNA was synthesized with oligo dt and Molony murine leukemia virus reverse transcriptase (GIBCO, Milano, Italy). Reverse transcription was performed at 37 °C for 50 min followed by an initial denaturation at 70 °C for 15 min.

PCR amplification was then performed with synthetic gene-specific primers (GENENCO, Firenze, Italy) for eNOS (forward primer, 5′-TGCACCCTTCCGGGGATTCT-3′; reverse primer, 5′-GGATCCCTGGAAAAGGCGGT-3′; product length, 189 bp).

Amplification was performed with 35 cycles of denaturation (95 °C for 30 s), annealing (62 °C for 30 s) and extension (72 °C for 30 s). Parallel amplification of rat glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was performed. The reaction was linear to 35 cycles with use of the ethidium bromide detection method. PCR products were separated by electrophoresis on a 2% agarose gel containing ethidium bromide and were visualized by ultraviolet-induced fluorescence. The intensity of each band was quantified using a densitometer. The resulting densities of the eNOS bands were expressed relative to the corresponding densities of the GAPDH bands from the same RNA sample (Hattori et al., 1997; Kobayashi et al., 1999).

2.3. Isolated aortic rings

2.3.1. Relaxant effects of acetylcholine and sodium nitroprusside

Segments of thoracic aorta obtained from the experimental groups of rats were cleaned of adherent connective tissue in Krebs–Henseleit solution and cut into rings (3–5 mm in length). The rings were carefully handled to avoid damage to the inner surface and suspended in organ bath chambers containing 10 ml Krebs–Henseleit solution of the following composition (mM): NaCl, 118; KCl, 2.8; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; and glucose, 5.5. The medium was gassed with a mixture of CO_2 (5%) and O_2 (95%) and maintained at 37 °C (pH 7.4). Tissues were connected via silk sutures to force–displacement transducers (model 7004; U. Basile, Comerio, VA, Italy), and changes in isometric force were displayed on a Gemini chart recorder (model 7070; U. Basile). All rings were gradually stretched to a basal resting tension of 1–1.5 g, which was maintained throughout the experiment; the preparations were allowed to equilibrate for 90 min. To evaluate maximal contraction, the tissues were depolarized with potassium chloride (KCl; 60 mM) and washed with Krebs–Henseleit solution. After 30 min, the aortic rings were contracted by norepinephrine (3×10^{-6} M), and when the contractile response was stabilized (steady-state phase, 12–15 min), relaxation was evaluated by cumulative addition of acetylcholine (from 10^{-10} to 10^{-4} M). The direct relaxant effect of the NO-donor sodium nitroprusside (cumulative concentrations 10^{-9}–10^{-5} M) was also recorded.

2.3.2. 6-Keto-$PGF_{1\alpha}$ release

After a suitable period of equilibration, the basal prostacyclin-releasing capacity of the aortic rings was measured

by evaluating the concentration of 6-keto-$PGF_{1\alpha}$, the stable metabolite of prostacyclin, in 1 ml of the bathing fluid after 20 min of incubation. The rate of release of 6-keto-$PGF_{1\alpha}$ was determined according to the enzyme immunoassay method (detection limit, 3 pg/ml) described by Pradelles et al. (1985), and was expressed as pg/mg wet tissue (pg/mg wt.).

2.4. Statistical analysis

Differences of data among groups in individual experiments were analyzed for statistical significance by one-way analysis of variance (ANOVA), followed by the Bonferroni test. A value of $P < 0.05$ was considered significant.

2.5. Drugs

The following drugs were used: N^G-monomethyl-L-arginine (Sigma); enalapril maleate (Merck Sharp & Dohme, New Jersey, USA); quinapril hydrochloride (Parke Davis, Lainate, Italy); norepinephrine chloride and acetylcholine chloride (Sigma); sodium nitroprusside (Merck, Darmstadt, Germany).

3. Results

3.1. Systolic blood pressure

Rats drinking water supplemented with L-NAME exhibited a progressive increase in systolic blood pressure from the second week on, reaching the value of 211 ± 2 mm Hg at week 8 (Table 1). Enalapril or quinapril in the drinking water significantly, and similarly, decreased systolic blood pressure (174 ± 1.9, L-NAME + enalapril; 176 ± 1.8 mm Hg, L-NAME + quinapril; $P < 0.05$ vs. L-NAME alone) (Table 1).

3.2. RT-PCR for aortic eNOS mRNA

In aortic tissues from L-NAME-treated rats, the levels of eNOS mRNA were significantly decreased as compared to those of control rats (− 46%, $P < 0.01$; Fig. 1). Treatment of these rats with enalapril or quinapril completely restored aortic eNOS mRNA levels (Fig. 1).

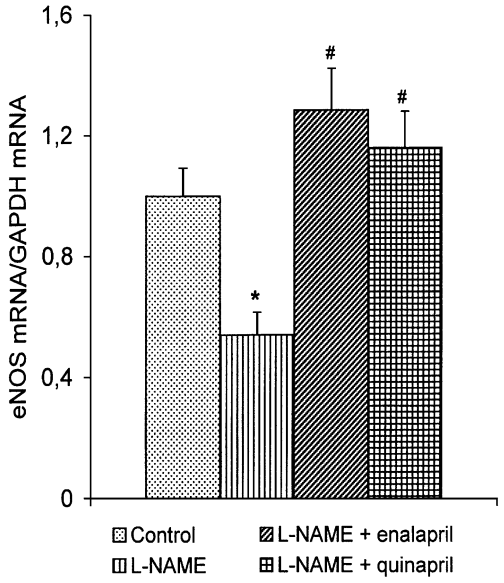

Fig. 1. eNOS mRNA expression in aortas from rats given L-NAME, L-NAME + enalapril, L-NAME + quinapril and in control rats. Total RNA was assayed by RT-PCR with gene-specific primers for eNOS and GAPDH. Values are expressed as means ± S.E.M. of five determinations. * $P < 0.01$ vs. control; # $P < 0.01$ vs. L-NAME.

3.3. Isolated aortic rings

3.3.1. Relaxant effect of acetylcholine and sodium nitroprusside

In this set of experiments, the contractions caused by norepinephrine (3×10^{-6} M) in aortic tissue prepared from untreated animals (1.78 ± 0.12 g over the resting tension), L-NAME (2.04 ± 0.16 g), L-NAME + enalapril (1.91 ± 0.12 g) or L-NAME + quinapril-treated rats (1.85 ± 0.14 g) were not significantly different, although a trend toward an increased reactivity to norepinephrine was found in aortic tissues from rats given L-NAME alone (data not shown). Exposure to cumulative concentrations of acetylcholine (from 10^{-10} to 10^{-4} M) resulted in a marked relaxation (93 ± 4%, maximal relaxant effect), expressed as percentage of norepinephrine-induced contractions of the aortic rings.

Table 1
Time-related changes in systolic blood pressure (mm Hg) in rats treated with L-NAME, L-NAME + enalapril, L-NAME + quinapril and in control rats

Group	Control	L-NAME	L-NAME + enalapril	L-NAME + quinapril
Basal	121.2 ± 2.9	120.0 ± 4.1	121.8 ± 2.4	126.2 ± 3.7
Second week	122.5 ± 2.6	167.8 ± 3.3[a]	171.2 ± 2.7[a]	168.7 ± 2.4[a]
Fourth week	124.3 ± 2.7	179.1 ± 3.1[a]	180.6 ± 2.3[a]	180.0 ± 1.8[a]
Sixth week	127.2 ± 2.0	196.2 ± 2.9[a]	197.5 ± 2.1[a]	197.8 ± 2.6[a]
Eighth week	129.3 ± 1.9	211.2 ± 2.6[a]	174.3 ± 1.9[a,b]	176.8 ± 1.8[a,b]

Data are mean ± S.E.M. of 10 animals.
[a] $P < 0.05$ vs. Control.
[b] $P < 0.05$ vs. L-NAME.

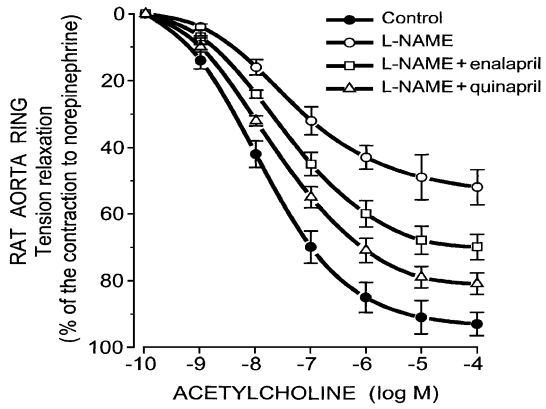

Fig. 2. Cumulative dose–response curves of acetylcholine in norepinephrine-precontracted aortic rings prepared from rats given L-NAME, L-NAME + enalapril, L-NAME + quinapril and in control rats. Points represent the mean values, and vertical bars, the S.E.M. of seven determinations. Statistical differences related to acetylcholine-induced maximal relaxation are: a vs. b, $P<0.001$; a vs. c and d, $P<0.05$; b vs. c and d, $P<0.01$.

In contrast, the sensitivity to acetylcholine of the aortic rings from rats given L-NAME alone was significantly reduced, corresponding only to 52 ± 5% (Fig. 2). When aortic tissues from rats given L-NAME + enalapril or L-NAME + quinapril were challenged with acetylcholine, the vasodilatation was almost fully restored, the maximal relaxant effect being 70 ± 4% and 81 ± 3%, respectively (Fig. 2).

Exposure of the aortic rings precontracted with norepinephrine to cumulative concentrations (from 10^{-9} to 10^{-5} M) of the nitrovasodilator sodium nitroprusside produced dose–response curves that were almost superimposable, except for a minimally reduced relaxation in the aortic rings from rats given L-NAME alone (Fig. 3).

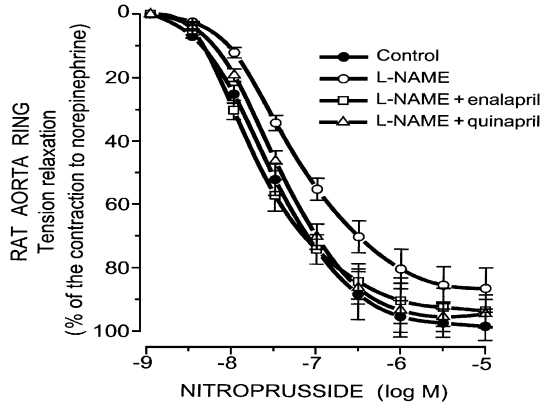

Fig. 3. Cumulative dose–response curves of sodium nitroprusside in norepinephrine-precontracted aortic rings prepared from rats treated with L-NAME, L-NAME + enalapril, L-NAME + quinapril and in control rats. Points represent the mean values, and vertical bars, the S.E.M. of seven determinations. P = NS.

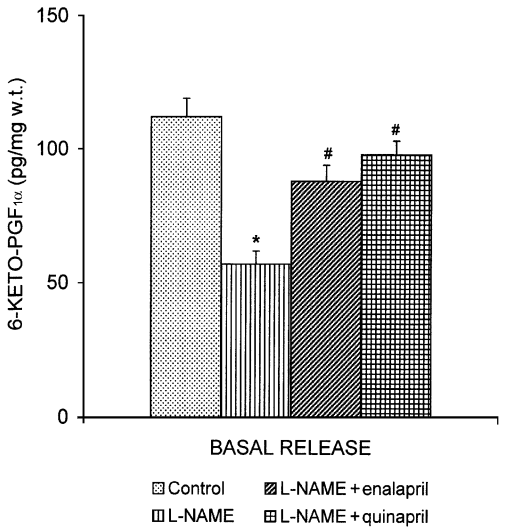

Fig. 4. Unstimulated release of 6-keto-prostacyclin ($PGF_{1\alpha}$) in 20 min from isolated aortic rings from rats treated with L-NAME, L-NAME + enalapril, L-NAME + quinapril and control rats. Values are expressed as means ± S.E.M. of seven determinations. * $P<0.01$ vs. control; # $P<0.05$ vs. L-NAME.

3.3.2. 6-Keto-$PGF_{1\alpha}$ release

The spontaneous release of 6-keto-$PGF_{1\alpha}$ into the bathing fluid of the aortic rings from the different experimental groups is reported in Fig. 4. Aortic segments of control rats released consistent amounts of the prostanoid (112 ± 7 pg/mg wt.), whereas the corresponding segments from rats given L-NAME alone exhibited a marked reduction in the release of 6-keto-$PGF_{1\alpha}$ (57 ± 5 pg/mg wt.) ($P<0.01$ vs. control). In the organ bath of the aortic rings from rats given L-NAME + enalapril or L-NAME + quinapril, the release of the prostacyclin metabolite was significantly increased (88 ± 6 and 98 ± 5 pg/mg wt., respectively; $P<0.05$ vs. L-NAME alone) (Fig. 4).

4. Discussion

In the present rat model of chronic NO inhibition, the endothelial vasodilator mechanism was severely impaired and the angiotensin-converting enzyme inhibitors enalapril and quinapril were capable to restore it. In fact, when the aortic rings from rats treated with L-NAME, an inhibitor of NO synthesis were precontracted with norepinephrine, acetylcholine lost a consistent part of its relaxant activity. The impairment in the endothelial vasodilator function was not related to changes in the sensitivity of vascular smooth muscle to NO since relaxation of the aortic tissues ensuing exposure to the NO-donor sodium nitroprusside was unchanged. A more likely explanation was that the impaired endothelial function in hypertensive rats given L-NAME alone mainly resulted from an impaired NOS function in the vascular endothelial cells. Consistent with this view, a

significant decrease of the eNOS mRNA expression was present in the aortic rings from rats given L-NAME. Addition of enalapril or quinapril to L-NAME rats decreased, although only partially (an event likely due to the short length of angiotensin-converting enzyme inhibitor treatment), the enhanced systolic blood pressure and improved the endothelium-dependent relaxation to acetylcholine exposure in the aorta. It is noteworthy that these findings were paralleled by a full recovery of eNOS mRNA expression in aortic tissues. There was no significant difference in the ability of the two angiotensin-converting enzyme inhibitors to restore endothelial vasodilator function and eNOS mRNA expression.

The present data on the presence of a dysfunction of the endothelial vasodilator mechanism in the aorta from L-NAME hypertensive rats broaden previous reports from Kung et al. (1995) and Henrion et al. (1996).

At variance with the pattern evidenced in large conduit arteries like aorta, reportedly, the resistance arteries, such as the mesenteric arteries, from L-NAME-treated rats did not have any attenuated response to acetylcholine following L-NAME treatment (Maeso et al., 1996; Navarro-Cid et al., 1996). This would confirm the idea that in large conduit arteries, endothelium-dependent relaxation is an NO-mediated event, whereas in resistance arteries, acetylcholine-induced relaxation rests mainly on other endothelium-derived relaxing factors (McCulloch et al., 1997).

The ability of either enalapril or quinapril to restore acetylcholine-induced dilatation in aortic rings from L-NAME hypertensive rats contrasts the reported failure of quinapril, given for 3 weeks to L-NAME-treated rats, to alter the blunted endothelial responses to acetylcholine (Henrion et al., 1996). However, our present findings are also validated by the ability of enalapril or quinapril to completely restore eNOS mRNA expression in the aortic tissues from L-NAME-treated rats. In this context, while the decreased functional activity of NOS in long-term L-NAME-treated rats is well established (Bertanova et al., 1999; Kobayashi et al., 2000), the effect on NOS activity of an "in vivo" treatment with angiotensin-converting enzyme inhibitors in L-NAME hypertensive rats is more controversial. Bertanova et al. (1999) and Pechanova et al. (1997) found no effect of captopril, given for 4 weeks on NOS inhibition in heart, aorta, kidney and brain, respectively, whereas Kobayashi et al. (2000) showed that a subdepressor dose of imidapril, given for 4 weeks to hypertensive rats, induced a significant increase of heart eNOS mRNA (Kobayashi et al., 2000). Our present results broaden Kobayashi's findings and showed that enalapril and quinapril were capable to restore eNOS mRNA in the aortic tissue from rats given L-NAME. The stimulatory effect of enalapril and quinapril on NOS might result from the known ability of angiotensin-converting enzyme inhibitors to inhibit kinase II, the enzyme that catalyzes the degradation of bradykinin (Erdos, 1977; Wiemer et al., 1991). The ensuing increase in bradykinin concentration, via stimulation of bradykinin B_2 receptor subtype (Linz and Scholkens, 1992; Bouaziz et al., 1994), would increase NOS activity.

Another noteworthy aspect of our study was the marked reduction in the release of 6-keto-$PGF_{1\alpha}$ from the aortic rings of L-NAME-treated rats. To the best of our knowledge, this is the first report of an impaired endothelial generation of prostacyclin in the hypertension model based on chronic L-NAME treatment. Up to now, the existence of a relationship between constitutively derived NO and cyclooxygenase products did not seem so clear. In rats treated chronically with L-NAME, a rise in the urinary levels of 6-keto-$PGF_{1\alpha}$, resulting in part from cyclooxygenase-2 expression in resistance mesenteric arteries, was reported (Henrion et al., 1997). In contrast, in cultured rat heart endothelial cells, the production of 6-keto-$PGF_{1\alpha}$ was, reportedly, stimulated by the NO donors SIN-1 (3-morpholino sydnonimine), GEA 3162 (3-aryl-substituted oxatriazole imine) and GEA 3175 (3-aryl-substituted oxatriazole sulfonyl chloride); L-NAME decreased the basal production of the prostanoid and this effect could be reversed by the NO donors (Sievi et al., 1997). In this vein, it is noteworthy that in a previous report (Rossoni et al., 1999), we demonstrated the presence in the aortic rings from growth hormone-deficient rats of an endothelial dysfunction due to an impaired NOS activity, which was also coupled with a decreased generation of prostacyclin (Rossoni et al., 1999). In sum, our present and previous results (Rossoni et al., 1999) support the in vitro findings from Sievi et al. (1997) and strongly imply an NO-induced stimulation of prostacyclin production, possibly, via cyclooxygenase activation. Hence, chronic NOS inhibition (our study) might impair endothelial prostacyclin production.

A contribution of the latter event to the endothelial dysfunction present in the L-NAME model of hypertension is envisaged. In the present study, both enalapril and quinapril restored prostacyclin production by endothelial aortic cells in L-NAME-treated rats. The protective action of the angiotensin-converting enzyme inhibitors was likely related to a decreased bradykinin inactivation, since bradykinin, reportedly, is effective to increase NO synthesis and to stimulate phospholipase activity (Wiemer et al., 1991; Linz et al., 1995).

In conclusion, L-NAME-induced hypertension in the rat is associated with a marked impairment of the endothelial vasodilator function depending primarily on a decreased NOS activity, with an ensuing contribution by a decreased endothelial production of prostacyclin. Enalapril and quinapril were equally effective to improve endothelial vasodilator function, prostacyclin endothelial production and to restore aortic eNOS mRNA.

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