Arterial function in nitric oxide-deficient hypertension: influence of long-term angiotensin II receptor antagonism

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Received 18 September 1998; accepted 23 November 1998

Abstract

Objective: Since the effects of angiotensin II receptor antagonism on arterial function in nitric oxide (NO)-deficient hypertension are unknown, we investigated the influence of losartan therapy (20 mg kg⁻¹ day⁻¹) on the control of arterial tone in N²-nitro-L-arginine methyl ester (L-NAME; 20 mg kg⁻¹ day⁻¹)-induced hypertension. Methods: Forty Wistar rats were divided into four groups: control, losartan, L-NAME, and losartan+L-NAME. The responses of isolated mesenteric arterial rings were examined in standard organ chambers after 8 treatment weeks. Results: Losartan therapy prevented the development of L-NAME-induced hypertension and the associated impairments of endothelium-independent relaxations to nitroprusside, isoprenaline, and cromakalim, vasodilators acting via the formation of NO, activation of β-adrenoceptors and opening of K⁺ channels, respectively. In addition, endothelium-dependent relaxations of noradrenaline-precontracted rings to acetylcholine during NO synthase inhibition in vitro were decreased in L-NAME rats, and clearly improved by losartan therapy. The inhibition of cyclooxygenase by diclofenac improved the responses to acetylcholine more effectively in L-NAME than losartan+L-NAME rats, but the relaxations remained decreased in L-NAME rats when compared with losartan+L-NAME rats. When hyperpolarization of smooth muscle was prevented by precontractions induced by high concentration of KCl, the responses to acetylcholine during combined NO synthase and cyclooxygenase inhibition were similar and almost abolished in all groups. Furthermore, superoxide dismutase, a scavenger of superoxide anions, enhanced the acetylcholine-induced relaxations more effectively in L-NAME than losartan+L-NAME rats, although plasma antioxidant capacity was similar in all study groups. Conclusion: Chronic L-NAME-induced hypertension was associated with attenuated arterial relaxation via endothelium-dependent and -independent mechanisms, both of which were improved by the losartan treatment. The mechanisms whereby losartan enhanced arterial relaxation in this model of experimental hypertension may have included enhanced hyperpolarization and increased sensitivity to NO in smooth muscle, and decreased vascular production of superoxide and vasoconstrictor prostanoids. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Antihypertensive agents; Endothelial factors; Hypertension; Nitric oxide; Smooth muscle

1. Introduction

The endothelial production of nitric oxide (NO) is essential for the maintenance of normal blood pressure [1]. Several disease states including essential hypertension have been associated with defects in the production or action of NO [2], which makes chronic NO-deficiency a particularly interesting model of hypertension. N²-nitro-L-arginine methyl ester (L-NAME) is a structural analogue of L-arginine and a potent competitive inhibitor of NO synthase (NOS) [3]. Accordingly, chronic administration of L-NAME has been shown to result in sustained hypertension in normotensive rats [4,5], with an associated impairment of endothelium-dependent arterial relaxations.
[6–9]. However, the findings concerning arterial responses to exogenous NO and various contractile agonists have been somewhat inconsistent in this model [6–8].

The angiotensin II (Ang II) receptor antagonist losartan exerts its antihypertensive action primarily through the competitive inhibition of Ang II binding to the AT₁ receptors. However, the antagonism of thromboxane A₂/prostaglandin H₂ receptors [10] and increased production of NO and vasodilatory prostanoids [11] have also been suggested to contribute to the long-term antihypertensive effects of losartan in experimental animals. Both losartan [12,13] and angiotensin converting enzyme (ACE) inhibitors [7,14] have been shown to prevent the development of the L-NAME-induced hypertension, and ACE-inhibitors have also been shown to improve endothelium-dependent relaxations [6,9] and prevent the attenuation of arterial contractile responses in this experimental model [7]. Nevertheless, the influence of Ang II receptor antagonist treatment on arterial function in L-NAME hypertensive rats has not been elucidated.

Since the inhibitory effect of orally administered L-NAME does not persist in vitro, the investigations on the control of arterial tone in L-NAME hypertension are somewhat difficult to perform [5]. The decline of NOS inhibition ex vivo could be counteracted by the addition of L-NAME to the organ bath, the approach of which, however, has not been applied in the previous reports. The present study was designed to examine the effects of long-term losartan-therapy on the control of arterial tone in L-NAME hypertensive Wistar rats, and the decline of NOS inhibition in vitro was eliminated by the addition of L-NAME to the bioassay. Special attention was paid to evaluate the roles of different endothelium-derived mediators in the vasodilator responses and to elucidate the possible functional changes in arterial smooth muscle.

2. Methods

2.1. Animals and experimental design

Male Wistar rats were housed four to a cage in an experimental animal laboratory (illuminated 06:00–18:00 h, temperature +22°C) with free access to drinking fluid and food pellets (Ewos, Södertälje, Sweden). The systolic blood pressures of the conscious animals were measured at endothelium is often confirmed by a clear relaxation to 1 h, temperature experimental animal laboratory (illuminated 06:00–18:00 force-displacement transducer and registered on a poly-22 4 4

added to the drinking fluid as follows: control (tap water); losartan (20 mg kg⁻¹ day⁻¹); L-NAME (20 mg kg⁻¹ day⁻¹); and losartan+L-NAME (doses of both losartan and L-NAME 20 mg kg⁻¹ day⁻¹). Drug solutions were prepared daily and given in light-proof bottles. L-NAME administration, losartan therapy and fortnightly blood pressure measurements were continued for 8 more weeks, whereafter the rats were anaesthetized by intraperitoneal administration of urethane (1.3 g kg⁻¹). The carotid artery was cannulated and blood samples were drawn into chilled tubes on ice containing 2.7 mmol EDTA. The plasma samples were centrifuged, and stored at −70°C until assayed. The hearts were removed and weighed, and the superior mesenteric arteries and small mesenteric artery segments were excised. The experimental design of the study was approved by the Animal Experimentation Committee of the University of Tampere, Finland, and the investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

2.2. Mesenteric arterial responses in vitro

The inhibitory effect of orally administered L-NAME on acetylcholine (ACH)-induced relaxation has been found to decline during successive responses in isolated arterial preparations from L-NAME-treated rats [5]. In preliminary experiments we also observed that arterial relaxation to ACH from L-NAME hypertensive rats improved during repeated challenges. We tested the in vitro concentrations of 0.1–100 μM L-NAME and found that 100 μM of L-NAME was needed to prevent the enhancement of relaxation to ACH during four repetitions. Therefore, the majority of the in vitro experiments were performed in the presence of 0.1 mM L-NAME as given below.

Five successive standard sections (3 mm in length) of the main superior mesenteric artery from each animal were cut, beginning 5 mm distally from the mesenteric artery–aorta junction. In the three most distal rings the endothelium was left intact, and from the first two pieces it was removed [15]. The rings were suspended in an organ bath chamber in physiological salt solution (PSS; pH 7.4) containing (in mM): NaCl 119.0, NaHCO₃ 25.0, glucose 11.1, CaCl₂ 1.6, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2. The PSS was aerated with 95% O₂ and 5% CO₂. The rings were equilibrated for 1 h at +37°C with a resting preload of 1.5 g, and contraction was measured with isometric force-displacement transducer and registered on a poly-22 4 4

graph (FT 03 transducer and Model 7 E Polygraph; Grass Instrument Co., Quincy, MA, USA). The presence of intact endothelium is often confirmed by a clear relaxation to 1 μM ACh in 1 μM noradrenaline (NA)-precontracted rings, and the absence of endothelium by the lack of this response [15]. However, in the present investigation the responses to ACh were almost completely absent in the L-NAME group. Therefore, no vascular preparations were excluded from the study.

2.2.1. Vascular preparation 1

Contractions of endothelium-denuded rings to KCl were
cumulatively determined. In solutions containing high concentrations of potassium (20–125 mM), NaCl was replaced with KCl on an equimolar basis. After the response the rings were rinsed with Ca\(^{2+}\)-free PSS, and, once the resting tension was restored, the rings were contracted twice with 10 \(\mu\)M NA in Ca\(^{2+}\)-free PSS to deplete the cellular Ca\(^{2+}\) stores. The rings were challenged with 125 mM KCl in Ca\(^{2+}\)-free PSS, whereafter Ca\(^{2+}\) was cumulatively added (0.01–2.5 mM) and the contraction registered [16]. Then the rings were rinsed with Ca\(^{2+}\)-free PSS and allowed a 20-min recovery period, and the same procedure was elicited in the presence of 0.5 mM nifedipine, and in the presence of nifedipine and 0.05 \(\mu\)M mibefradil. All responses were performed in the presence of 0.1 mM L-NAME.

2.2.2. Vascular preparation 2

Relaxations to isoprenaline, cromakalim and nitroprusside (NP) were cumulatively examined in endothelium-denuded rings precontracted with 1 \(\mu\)M NA. Thereafter, the relaxations to NP were examined in rings precontracted with 50 mM KCl. The rings were allowed a 30 min equilibration period in PSS between the response to each vasodilator. The responses in the L-NAME and losartan+ L-NAME groups were performed in the presence of 0.1 mM L-NAME.

2.2.3. Vascular preparation 3

Contractions to NA were determined in endothelium-intact rings in the presence of 0.1 mM L-NAME. The responses to NA were also elicited in the presence of L-NAME and 3 \(\mu\)M diclofenac. The rings were allowed a 30 min equilibration period in PSS containing the above inhibitors between the two determinations.

2.2.4. Vascular preparation 4

Relaxations to ACh in the presence of 0.1 mM L-NAME, and in the presence of L-NAME and 3 \(\mu\)M diclofenac were examined in endothelium-intact rings precontracted with 1 \(\mu\)M NA. The responses to ACh in the presence of L-NAME plus 3 \(\mu\)M diclofenac were also elicited in rings precontracted with 50 mM KCl. The rings were allowed a 30 min equilibration in PSS containing the above inhibitors between each cumulative relaxation.

2.2.5. Vascular preparation 5

Relaxations to ACh in the presence of 0.1 mM L-NAME were examined in endothelium-intact arterial rings precontracted with 1 \(\mu\)M NA. The responses to ACh were also elicited in the presence of L-NAME plus 50 U/ml superoxide dismutase (SOD), and in the presence of L-NAME, SOD plus 100 U/ml catalase. The rings were allowed a 30 min equilibration period in PSS containing the above enzyme(s) between each cumulative relaxation.

2.3. Morphological studies

Third order mesenteric arterial branches were chosen from the vascular bed that feeds the small intestine 2–6 cm prior to the ileocecal junction. A segment (2 mm in length) of the mesenteric artery was isolated under a dissection microscope (Nikon SMZ-2T, Nikon Inc., Japan) and transferred to the myograph chamber (Living Systems Instrumentation Inc., Burlington, VT, USA) containing 8 ml of PSS (pH 7.4) of the following composition (mM): NaCl 119.0, NaHCO\(_3\) 25.0, glucose 11.1, CaCl\(_2\) 2.5, KCl 4.7, KH\(_2\)PO\(_4\) 1.2, MgSO\(_4\) 1.2, Na\(_2\)EDTA 0.4. The proximal end of the vessel was cannulated with a micropipette and flushed to remove the remaining blood before the cannulation of the distal end. In the beginning of the study the arterial wall thickness and lumen diameter of the unpressurized vessels were recorded by the use of a video monitoring system (Video dimension analyzer, Living Systems Instrumentation Inc., Burlington, VT, USA). The artery was superfused at a rate of 10 ml min\(^{-1}\) and aerated with 95\% O\(_2\) and 5\% CO\(_2\). Thereafter the intraluminal pressure was slowly raised to 60 mmHg. The pressure in the proximal end of the artery segment was monitored by a pressure transducer and controlled by a servo perfusion system (Pressure servo control, Living Systems Instrumentation Inc., Burlington, VT, USA). After the vessel had equilibrated for 40 min in 60 mmHg the arterial dimensions were recorded.

2.4. Total peroxyl radical-trapping and vitamin E

Total peroxyl radical-trapping capacity was determined by the chemiluminescence method which has been described elsewhere [17]. Briefly, the production of peroxyl radicals by ABAP (2,2'-azobis[2-amidinopropane] hydrochloride) induces luminol-enhanced chemiluminescence, and the time during which the added test sample extinguishes the reaction is directly proportional to its peroxyl radical-trapping antioxidant capacity. The test reaction was initiated by mixing 475 \(\mu\)l of oxygen-saturated sodium phosphate buffer (100 mM, pH 7.4) with 50 \(\mu\)l of 400 mM ABAP (prepared in 100 mM phosphate-buffer) and 50 \(\mu\)l of 10 mM luminol in 20 mM boric acid–Borax-buffer in a cuvette (pH 9.0). The cuvette was placed in a luminometer (37°C), and the plasma sample (25 \(\mu\)l) was injected after the luminescence had stabilized, whereafter the chemiluminescence was measured at 40 s intervals. The watersoluble tocopherol was used as a standard. Vitamin E concentration was determined by modified high performance liquid chromatography (HPLC) [18], and in our modification the UV detection of antioxidants was replaced by an LC-4 amperometric detector (Bioanalytical Systems Inc. West Lafayette, USA).
2.5. Data presentation and analysis of results

The maximal contractile responses to NA and KCl were expressed in grams. The $EC_{50}$ for NA and KCl as well as $EC_{25}$ for calcium in each ring was calculated as a percentage of maximal response and presented as the negative logarithm ($pD_{50}$ and $pD_{25}$, respectively), which values were also used in the statistical analysis. The $pD_{25}$ values were chosen for the statistics in the contractile responses to cumulative calcium, since the responses did not reach the level of 50 per cent in the presence of the inhibitors. The relaxations in response to ACh, NP, isoprenaline and cromakalim were presented as a percentage of pre-existing contractile force.

Statistical analysis was carried out by one-way analysis of variance (ANOVA) supported by the Bonferroni test when carrying out pairwise comparisons between the test groups. ANOVA for repeated measurements was applied for data consisting of repeated observations at successive time points. Unless otherwise indicated the $P$ values in the text refer to ANOVA for repeated measurements. All results are expressed as mean±SEM, and the differences were considered significant when $P<0.05$.

2.6. Drugs

The following drugs were used: losartan potassium (Merck Pharmaceutical Company, Wilmington, DE, USA), acetylcholine chloride, catalase, cromakalim, isoprenaline hydrochloride, $N^\text{G}$-nitro-$L$-arginine methyl ester hydrochloride, superoxide dismutase (Sigma Chemical Co, St Louis, MO USA), $L$-noradrenaline $L$-hydrogentartrate, nitroprusside (Fluka Chemie AG, Buchs SG, Switzerland), diclofenac (Voltaren injection solution, Ciba-Geigy, Basel, Switzerland) and nifedipine (Orion Pharma Ltd., Espoo, Finland). For the preparation of stock solutions the compounds used in the in vitro studies were dissolved in distilled water, with the exception of cromakalim and nifedipine (in 50% ethanol). All solutions were freshly prepared before use and protected from light. Losartan and $N^\text{G}$-nitro-$L$-arginine methyl ester hydrochloride were dissolved directly in the drinking water of the rats.

3. Results

3.1. Blood pressure, body and heart weights, plasma total peroxyl radical-trapping and vitamin E

Long-term administration of L-NAME resulted in an elevation of blood pressure which reached its maximum within 4 weeks, while the losartan treatment effectively prevented the development of hypertension. There was no significant difference in the systolic blood pressure of the control group when compared with the losartan group during the follow-up period (Fig. 1). The body weights were corresponding in all groups. The heart–body weight ratios were comparable in L-NAME and control groups, whereas the losartan therapy reduced relative heart weights in both of these groups. Plasma total peroxyl radical-trapping capacities and vitamin E concentrations were comparable between the study groups (Table 1).

3.2. Endothelium-independent relaxations of the mesenteric artery

In order to make proper interpretations from the endothelium-dependent relaxations, the vasodilatory properties of arterial smooth muscle were examined. The relaxations of endothelium-denuded NA-precontracted rings to NP, isoprenaline and cromakalim, the vasodilators acting via the formation of NO, activation of $\beta$-adrenoceptors and opening of ATP-sensitive $K^+$ channels ($K_{ATP}$), respectively, were impaired in L-NAME rats when compared with all other groups. All of these impairments in arterial relaxation were clearly improved by the losartan treatment. In addition, when hyperpolarization of smooth muscle was prevented by precontractions with 50 mM KCl [19], the relaxations to NP were still impaired in L-NAME rats and normalized by losartan (Fig. 2).

3.3. Endothelium-dependent relaxations

The relaxations induced by ACh in NA-precontracted arterial rings in the presence of L-NAME were markedly impaired in the L-NAME group when compared with the control group, while these responses were clearly improved by losartan treatment in both of these groups (Fig.
Table 1
Experimental group data at close of the study

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Losartan</th>
<th>L-Name</th>
<th>Los+L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>432±16</td>
<td>432±13</td>
<td>440±11</td>
<td>450±11</td>
</tr>
<tr>
<td>Heart weight (mg)</td>
<td>1148±27</td>
<td>1047±34*</td>
<td>1234±24*</td>
<td>1095±16*</td>
</tr>
<tr>
<td>Heart–body weight ratio (mg g⁻¹)</td>
<td>2.7±0.1</td>
<td>2.4±0.1*</td>
<td>2.8±0.1</td>
<td>2.4±0.1*</td>
</tr>
<tr>
<td>Total peroxyl radical-trapping (µM)</td>
<td>624±54</td>
<td>570±33</td>
<td>594±41</td>
<td>545±20</td>
</tr>
<tr>
<td>Vitamin E (µM)</td>
<td>18.2±0.9</td>
<td>15.0±0.9</td>
<td>16.1±1.5</td>
<td>17.1±1.7</td>
</tr>
</tbody>
</table>

Values are mean±SEM, n=7–10 for all groups.
* P<0.05 compared with control group, † P<0.05 compared with L-NAME group (Bonferroni test).

3A). The addition of the cyclooxygenase (COX) inhibitor diclofenac to the organ bath improved the relaxations to ACh in all other groups (P<0.05), but not in the losartan group, and abolished the difference between the control and losartan groups, while the relaxations still remained impaired in the L-NAME group when compared with the other groups. On the other hand, diclofenac caused a 5.2-fold increase in the maximum relaxation to ACh in the losartan+L-NAME group when compared with the 1.6-fold change in the losartan+L-NAME group (P<0.05, one-way ANOVA) (Fig. 3B). The responses to ACh were almost abolished in all groups when induced in KCl-precontracted rings in the presence of L-NAME and diclofenac (Fig. 3C).

When SOD was added to the organ bath the relaxations to ACh were enhanced in all other groups (P<0.05), but not in the losartan group, while the responses to ACh remained impaired in the L-NAME group when compared with the other groups (Fig. 3D). However, the addition of SOD caused a 3.4-fold increase in the maximum response

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Fig. 2. Line graphs show relaxations to nitroprusside after precontraction with 1 µM noradrenaline (A) and 50 mM KCl (B), and relaxations to isoprenaline (C) and cromakalim (D) after precontraction with 1 µM noradrenaline in endothelium-denuded mesenteric arterial rings. The groups were control (○), losartan (□), L-NAME (●), and losartan+L-NAME (■). L-NAME in vitro (0.1 mM) was present in the two latter groups, values represent mean±SEM, n=8–10 in each group; * P<0.05, ANOVA for repeated measurements.
to ACh in the L-NAME group when compared with the 1.5-fold change in the losartan+L-NAME group \((P<0.05\text{, one-way ANOVA})\). In addition, SOD abolished the difference in response to ACh between the control and losartan group (Fig. 3D). The further addition of catalase had no significant effects on the relaxation to ACh in any of the study groups (data not shown).

3.4. Vasoconstrictor responses

The contractile experiments were performed to elucidate the possible differences in vasoconstriction which may curtail the results on arterial relaxation. The maximal contractile force generation to NA in the presence of L-NAME was comparable in control, L-NAME, and losartan+L-NAME groups, while the maximal contraction was lower in losartan group (Table 2). The vasoconstrictor sensitivity (i.e. \(pD_{50}\) values) to NA in the presence of L-NAME, and in the presence of L-NAME and diclofenac, was similar in all study groups. In addition, the arterial rings of the control, losartan, and L-NAME groups showed similar contractile force generation and sensitivity to KCl, whereas arterial contractile sensitivity, but not the maximal contractile force, was somewhat higher in losartan+L-NAME group (Table 2).

The contractile sensitivity of arterial smooth muscle (i.e. \(pD_{25}\) values) to the cumulative addition of Ca\(^{2+}\) during depolarization with KCl was similar in all study groups (Table 2). Moreover, the L-type Ca\(^{2+}\) entry blocker nifedipine equally decreased arterial contractile sensitivity to Ca\(^{2+}\) in the control, losartan, and L-NAME groups, while the effect of nifedipine was smaller in the losartan+L-NAME group. In contrast, the T-type Ca\(^{2+}\) entry blocker mibefradil, when added after nifedipine, reduced the Ca\(^{2+}\) sensitivity of arterial smooth muscle more effectively in the L-NAME group than in the other groups. Losartan treatment was without effect on the inhibitory effect of mibefradil on the Ca\(^{2+}\) sensitivity in either group (Table 2).

3.5. Morphological studies

The wall thickness and wall to lumen ratio of the mesenteric resistance arteries from L-NAME rats were higher than in those from control rats in both pressurized and unpressurized arteries. The wall thickness and wall to
### Table 2
Parameters of contractile responses of isolated mesentric arterial rings in the experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Losartan</th>
<th>L-NAME</th>
<th>Los + L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Noradrenaline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pD50 (–log M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+E with L-NAME</td>
<td>6.802±0.093</td>
<td>6.910±0.166</td>
<td>6.728±0.113</td>
<td>7.017±0.226</td>
</tr>
<tr>
<td>+E with L-NAME and diclofenac</td>
<td>6.286±0.120</td>
<td>6.209±0.141</td>
<td>5.981±0.081</td>
<td>6.188±0.203</td>
</tr>
<tr>
<td>Maximal force (g)</td>
<td>2.532±0.264</td>
<td>1.735±0.257*</td>
<td>2.473±0.222</td>
<td>2.457±0.138</td>
</tr>
<tr>
<td><strong>Potassium chloride</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pD50 (–log M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–E</td>
<td>1.417±0.018</td>
<td>1.426±0.020</td>
<td>1.403±0.016</td>
<td>1.474±0.028*</td>
</tr>
<tr>
<td>Maximal force (g)</td>
<td>2.597±0.233</td>
<td>2.028±0.330</td>
<td>2.068±0.212</td>
<td>2.310±0.172</td>
</tr>
<tr>
<td><strong>Calcium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pD25 (–log M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–E</td>
<td>3.969±0.056</td>
<td>3.989±0.090</td>
<td>3.927±0.064</td>
<td>3.866±0.086</td>
</tr>
<tr>
<td>–E with nifedipine</td>
<td>3.444±0.086</td>
<td>3.534±0.069</td>
<td>3.646±0.097</td>
<td>3.757±0.107*</td>
</tr>
<tr>
<td>–E with nifedipine and mibebradil</td>
<td>3.207±0.091</td>
<td>3.171±0.085</td>
<td>3.113±0.088</td>
<td>3.347±0.112</td>
</tr>
<tr>
<td>Change in pD25 value induced by nifedipine</td>
<td>–0.525±0.105</td>
<td>–0.456±0.075</td>
<td>–0.281±0.105</td>
<td>–0.110±0.080*</td>
</tr>
<tr>
<td>mibebradil</td>
<td>–0.207±0.082</td>
<td>–0.345±0.130</td>
<td>–0.533±0.068*</td>
<td>–0.409±0.098</td>
</tr>
</tbody>
</table>

Values are mean±SEM, n=8–10 for all groups.
+E and –E, endothelium intact and endothelium-denuded arterial rings, respectively.
pD50 and pD25 are negative logarithms of the concentration of agonist producing 50% and 25% of maximal contractile force, respectively.
* P<0.05 compared with control group, † P<0.05 compared with L-NAME group (Bonferroni test).

The lumen ratio of the losartan+L-NAME rats did not differ from those of the control rats when the measurements were performed without intraluminal pressure. However, the effect of losartan on wall thickness and wall to lumen ratio was absent when the dimensions were measured under 60 mmHg of pressure. No differences were observed in the external diameter of pressurized and unpressurized arteries between the study groups (Table 3).

### 4. Discussion

Chronic inhibition of NOS is a new model of hypertension [4]. In this study L-NAME administration resulted in a marked hypertension which reached its maximum within 4 weeks, whereas losartan therapy totally prevented the elevation of blood pressure, which agrees with previous experiments on ACE inhibitors [7,14,20] and losartan [13,21]. In addition, losartan has even been shown to reverse established L-NAME hypertension in rats [22]. However, the present results for the first time showed that the impairments of endothelium-dependent and -independent arterial relaxation associated with NO-deficient hypertension can effectively be antagonized by chronic Ang II receptor antagonism.

The heart weight–body weight ratios did not differ between the L-NAME and control rats in this study, which agrees with some previous reports [12,23], although contradictory findings have been published [14,24]. Despite the absence of cardiac hypertrophy in the L-NAME rats,
losartan therapy reduced heart weights in this study. Previously, the stimulation of AT$_1$-receptors has been shown to exert an antigrowth effect on rat cardiomyocytes [25], which could explain the effect of losartan on heart weights, since AT$_1$-receptor antagonism leads to the accumulation of Ang II, which results in increased stimulation of the unopposed AT$_1$-receptors [26,27].

We found that oral L-NAME induced arterial hypertrophy in the mesenteric resistance vessels, which is in concert with previous reports [5,24,28]. In the spontaneously hypertensive rat (SHR) losartan has been reported to decrease arterial hypertrophy when microscopic examinations were performed in paraffin-embedded preparations [29], and the present measurements in unpressurized arteries yielded parallel results. However, the measurements in pressurized arteries, which represent a more physiological state, did not show differences between the L-NAME and losartan+L-NAME groups. Thus, losartan therapy did not reduce arterial hypertrophy in the L-NAME rats even though it normalized their blood pressure. Therefore, vascular hypertrophy in this model of hypertension appears to be independent of blood pressure, and may result from decreased vascular NO production, since endothelium-derived NO has been shown to inhibit smooth muscle growth [30].

The arterial relaxations induced by the NO-donor nitroprusside have been suggested to be enhanced [7,8], or remain unaffected in L-NAME hypertension [6]. However, in our study the L-NAME rats showed attenuated relaxations to nitroprusside in both NA- and KCl-precontracted vascular rings, suggesting that the sensitivity of arterial smooth muscle to NO was decreased. In addition, the relaxations induced by the β-adrenoceptor agonist isoprenaline and the K$_{ATP}$ opener cromakalim were also impaired in the L-NAME rats. Therefore, L-NAME hypertension was associated with attenuated vasorelaxant responses via cGMP, cAMP and the opening of K$^+$ channels, which suggests a general impairment of relaxations in arterial smooth muscle. Since all these impairments have also been described in SHR [31], they are likely to result from the long-term elevation of blood pressure. This view is supported by the fact that the present antihypertensive effect of losartan was accompanied by a complete normalization of these changes.

ACh dilates arteries via the release of several factors from the endothelium, the most prominent of these being NO, prostacyclin (PGI$_2$) and the endothelium-derived hyperpolarizing factor (EDHF) [32]. Arterial relaxations to ACh have been shown to be impaired in L-NAME hypertensive rats [6–8,20], and trandolapril has been suggested to prevent this impairment [6,20]. Furthermore, flow-induced dilatation has been reported to be attenuated in L-NAME hypertensive, the attenuation of which was prevented by quinapril [9]. In agreement with these findings, the relaxations of arterial rings to ACh were markedly impaired in the present L-NAME rats, and effectively antagonized by concomitant losartan therapy. Moreover, losartan improved endothelium-mediated vasodilation in the normotensive control rats, a finding which has also been observed with ACE inhibitors [33].

The chemical antagonism between superoxide and NO is recognized as an important modulator of vascular tone. In addition, superoxide has been reported to inhibit the vascular synthesis of PGI$_2$ without affecting that of the vasoconstrictor thromboxane A$_2$ [34]. Therefore, superoxide production or decreased antioxidant activity in the cardiovascular system could increase arterial tone and contribute to the development of hypertension. In the present study, the total peroxyl radical-trapping capacity and vitamin E concentration in plasma were comparable in all groups, suggesting that L-NAME hypertension is not associated with changes in circulating antioxidant capacity. In contrast, losartan treatment appeared to reduce the production of superoxide in the arteries of L-NAME rats, since the enhancing effect of SOD, the superoxide anion scavenger, on the relaxations to ACh was more pronounced in the L-NAME group when compared with the losartan+L-NAME group. Moreover, the difference in the relaxation induced by ACh between the control and losartan group was abolished by SOD, suggesting that losartan therapy reduced the production of superoxide also in control rats. The view of reduced superoxide production by losartan is in agreement with previous results whereby losartan-treatment decreased the production of superoxide and normalized the ACh-induced arterial relaxations in Ang II hypertensive rats [35].

The production of vasoconstrictor prostanoids has been suggested to contribute to the impaired endothelium-mediated vasodilatation in L-NAME hypertension [6]. In this study, the inhibition of COX by diclofenac clearly enhanced the relaxations to ACh in the L-NAME and losartan+L-NAME groups, suggesting that COX-derived contractile factors were involved in these responses. Moreover, losartan appeared to reduce the production of these factors in L-NAME rats, since the shift in the relaxation to ACh following diclofenac was more pronounced in the L-NAME group when compared with the losartan+L-NAME group. The release of vasoconstrictor prostanoids was probably also reduced in the control rats by losartan, since the difference in the relaxations to ACh between the control and losartan groups was abolished by diclofenac. It is noteworthy that decreased superoxide production may also have contributed to the enhanced endothelium-mediated vasodilatation after diclofenac administration, since COX is a significant source of superoxide [34].

The endothelium-dependent relaxations which are resistant to both NOS and COX inhibitions are probably mediated by EDHF [36]. The chemical characteristics of EDHF are unknown, but functionally this factor is a K$^+$ channel opener [36], the action of which can be inhibited by K$^+$ channel blockers or by cell membrane depolarization with high K$^+$ concentration [19]. Although all present
study groups showed distinct NOS and COX inhibitor-resistant relaxations to ACh, the remaining responses in the L-NAME group were attenuated when compared with all others, while the responses in the losartan+L-NAME group did not differ from the control group. Thus, losartan prevented the impairment of endothelium-dependent hyperpolarization in L-NAME-treated rats. The precontraction of arterial rings with KCl almost abolished the remaining NOS and COX inhibitor-resistant relaxations to ACh, suggesting that these responses were mediated by EDHF. It is noteworthy that NO has been shown to inhibit the production and action of EDHF [37,38]. Therefore, in pathophysiological states like hypertension, which may be associated with decreased bioavailability of NO, EDHF-mediated vasorelaxation could be of greater importance than under normal conditions [37]. Nevertheless, decreased endothelium-dependent hyperpolarization has been reported in genetic, renal, and mineralocorticoid-NaCl forms of experimental hypertension [39–41], and the present results suggest that the same also holds true for L-NAME hypertension.

Impaired endothelium-dependent hyperpolarization could result from reduced sensitivity of smooth muscle to EDHF or from decreased endothelial release of EDHF. The present results whereby the relaxations induced by the $K_{ATP}$ opener cromakalim were attenuated in L-NAME rats suggest that the sensitivity of smooth muscle to hyperpolarizing factors was decreased. In addition, isoprenaline has been reported to hyperpolarize arterial smooth muscle via $K_{ATP}$ and $K_{Ca}$ [42,43]. Thus, the present finding whereby isoprenaline-induced relaxation was impaired in L-NAME rats is also in agreement with the view of reduced hyperpolarization of smooth muscle in these rats.

The present study confirmed the earlier finding whereby L-NAME hypertension does not affect vasoconstrictor responses to NA [6]. Losartan treatment somewhat reduced maximal contractile force generation to NA in control rats, but was without effect on the responses to NA in L-NAME rats. Previously, a 3-week administration of L-NAME has been reported to decrease arterial contractions to KCl in rats [7,8], but we observed no differences between the L-NAME and control rats in the contractions induced by KCl in this 8-week study. The constrictor sensitivity of arterial smooth muscle to cumulative addition of Ca$^{2+}$ was also comparable in L-NAME and control rats, and the response remained unaffected by losartan. Previously, the arteries of SHR have been reported to be more sensitive to L-type Ca$^{2+}$ entry blockade by nifedipine than those of Wistar–Kyoto rats [16]. In this study, the L-NAME rats did not show increased sensitivity to the action of nifedipine, while the T-type Ca$^{2+}$ entry blocker mibebradil elicited a more pronounced shift in the Ca$^{2+}$ sensitivity of arterial rings from L-NAME rats, which suggests increased Ca$^{2+}$ influx via T-type channels in this model of experimental hypertension.

In conclusion, chronic L-NAME hypertension was asso-

## References


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