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Losartan and enalapril therapies enhance vasodilatation in the mesenteric artery of spontaneously hypertensive rats

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Abstract

We studied the effects of 10-week long enalapril and losartan treatments (4 and 15 mg kg^{-1} day^{-1}, respectively) on mesenteric arterial function in vitro in spontaneously hypertensive rats (SHR) and Wistar–Kyoto rats (WKY). The relaxations of noradrenaline-precontracted rings to acetylcholine, nitroprusside and cromakalim were similar in WKY and enalapril- and losartan-treated SHR, and more pronounced than in untreated SHR. The responses to acetylcholine were attenuated by N^G-nitro-L-arginine methyl ester in WKY and drug-treated SHR, but were completely inhibited in untreated SHR. When hyperpolarization of smooth muscle was prevented by KCl-induced precontractions, no differences were found in the relaxations to acetylcholine and nitroprusside between the groups, and the dilatations to cromakalim were abolished. Moreover, in noradrenaline-precontracted rings of drug-treated SHR, the addition of tetraethylammonium attenuated the nitric oxide synthase and cyclooxygenase-resistant relaxations to acetylcholine and abolished the enhanced dilatations to nitroprusside. In conclusion, since the enhancement of vasorelaxation in enalapril- and losartan-treated SHR was abolished by conditions preventing hyperpolarization, the improved vasodilatation following these therapies could be attributed to enhanced vasodilatation via K^+ channels in this model of hypertension.

Keywords: Angiotensin converting enzyme inhibition; Angiotensin receptor antagonism; Smooth muscle, arterial; Endothelium; Hyperpolarization; Spontaneously hypertensive rat (SHR); Wistar–Kyoto rat (WKY)

1. Introduction

The antihypertensive action of angiotensin converting enzyme inhibitors is primarily based on the inhibition of angiotensin II formation, while angiotensin II antagonists compete with angiotensin II at the angiotensin AT_1 receptor. However, endogenous nitric oxide (NO) and prostaglandins have also been reported to contribute to the long-term antihypertensive effects of angiotensin II antagonist and angiotensin converting enzyme inhibitor therapies in experimental animals (Cachofeiro et al., 1995). In addition, both of these therapies have been shown to augment endothelium-mediated arterial relaxation in experimental hypertension (Gohlke et al., 1993; Soltis, 1993; Tschudi et al., 1994; Rodrigo et al., 1997), and angiotensin converting enzyme inhibitor treatment has even been found to improve endothelium-mediated dilatation in hypertensive humans (Schiffrin and Deng, 1995).

The improved endothelial function following angiotensin converting enzyme inhibition has been attributed to increased release of NO (Berkenboom et al., 1995), reduced release of contractile factors from the endothelium (Hutri-Kähönen et al., 1997; Rodrigo et al., 1997),

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augmented endothelium-dependent hyperpolarization (Kähönen et al., 1995) and enhanced formation of vasodilatory prostaglandins (Schrör, 1990). These effects, however, do not appear to be solely related to reduced angiotensin II generation (Sunman and Sever, 1993), and specially diminished degradation of bradykinin has been implicated in the beneficial cardiovascular effects of angiotensin converting enzyme inhibitors (Linz et al., 1995). In contrast to angiotensin converting enzyme inhibition, the role of different mediators in the augmented endothelium-dependent dilatation following long-term angiotensin II antagonism has not been studied in detail.

The present study was designed to examine the effects of long-term therapy with losartan and enalapril on the control of arterial tone in spontaneously hypertensive rats (SHR) and normotensive Wistar–Kyoto rats (WKY). Special attention was paid to evaluate the role of hyperpolarizing mechanisms in endothelium-dependent and -independent dilatory responses. This study confirmed earlier findings whereby treatment with either angiotensin converting enzyme inhibitor or angiotensin receptor antagonist enhanced endothelium-mediated dilatation. However, the present results for the first time showed that improved arterial dilatation following these treatments could be attributed to enhanced vasodilatation via K^+ channels.

2. Materials and methods

2.1. Animals and experimental design

Male SHR (Okamoto–Aoki strain) and age-matched WKY were obtained from Møllegaard's Breeding Centre, Ejby, Denmark. The animals were housed in an experimental animal laboratory (illuminated 0600–1800 h, temperature +22°C) with free access to water and chow (Ewos, Södertälje, Sweden). The systolic blood pressures of conscious animals were measured at +28°C by the tail-cuff method (Model 129 Blood Pressure Meter; IITC, Woodland Hills, CA, USA). At 7 weeks of age, both SHR and WKY were divided into three groups of equal mean systolic blood pressures. Thereafter, SHR ($n = 10$) and WKY ($n = 10$) were given enalapril, while the other SHR ($n = 10$) and WKY ($n = 10$) groups received losartan in drinking water in light-proof bottles, (average dose 4 and 15 mg kg^{-1} day^{-1}, respectively, fresh drug solutions being daily prepared), whereas untreated SHR ($n = 15$) and normotensive WKY ($n = 15$) were kept on normal drinking fluid. Enalapril and losartan therapies and blood pressure measurements were continued for 10 weeks, whereafter the drug administrations were withdrawn 1 day before the rats were anaesthetized by intraperitoneal administration of urethane (1.3 g kg^{-1}) and exsanguinated. The hearts were removed and weighed, and the superior mesenteric arteries excised. The experimental design of the study was approved by the Animal Experimentation Committee of the University of Tampere, Finland. Moreover, the investigation complies with the European Community guidelines for the use of experimental animals.

2.2. Mesenteric arterial responses in vitro

Five successive standard sections (3 mm in length) of the 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, vascular endothelium was gently removed (Arvola et al., 1992). The rings were placed between stainless steel hooks (diameter 0.3 mm) and suspended in an organ bath chamber (volume 20 ml) in physiological salt solution (PSS; pH 7.4) of the following composition (mM): NaCl 119.0, $NaHCO_3$ 25.0, glucose 11.1, $CaCl_2$ 1.6, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, and aerated with 95% O_2 and 5% CO_2. The rings were initially equilibrated for 1 h at +37°C with a resting tension of 1.5 g. The force of contraction was measured with an isometric force–displacement transducer and registered on a polygraph (FT 03 transducer and Model 7 E Polygraph; Grass Instrument, Quincy, MA, USA). The presence of intact endothelium in vascular preparations was confirmed by a clear relaxation response to 1 μM acetylcholine in 1 μM noradrenaline-precontracted rings, and the absence of endothelium by the lack of this response. If any relaxation was seen in endothelium-denuded rings, the endothelium was further rubbed.

2.3. Endothelium-dependent relaxations after receptor-mediated precontraction

Relaxation responses to acetylcholine were examined in endothelium-intact mesenteric arterial rings precontracted with 1 μM noradrenaline, which resulted in approximately 60% of the maximal contractile response attained in each group. The responses to acetylcholine were also elicited in the presence of 3 μM diclofenac; in the presence of diclofenac and 0.1 mM N^G-nitro-L-arginine methyl ester (L-NAME); and in the presence of diclofenac, L-NAME and 1 mM tetraethylammonium. The next concentration of relaxing compound was added only after the previous level of relaxation was stable. The rings were allowed a 30 min equilibration period in PSS (containing the above agents) between each relaxant response.

2.4. Endothelium-dependent relaxations after depolarization-mediated precontraction

Relaxations to acetylcholine were examined in endothelium-intact rings precontracted with 50 mM KCl. The responses to acetylcholine were repeated in the presence of 3 μM diclofenac and 0.1 mM L-NAME.

2.5. Receptor-mediated contractions

Concentration–response curves of endothelium-intact rings to noradrenaline were cumulatively determined. The contractions to noradrenaline were then elicited in the presence of 3 μM diclofenac and 0.1 mM L-NAME.

2.6. Endothelium-independent relaxations to isoprenaline and cromakalim

Relaxations to isoprenaline and cromakalim were examined in endothelium-denuded rings precontracted with 1 μM noradrenaline and after precontraction with 50 mM KCl.

2.7. Arterial contractions to KCl and endothelium-independent relaxations to nitroprusside

Concentration–response curves for KCl were determined in endothelium-denuded rings. Thereafter, relaxations to nitroprusside were examined in rings precontracted with 1 μM noradrenaline as well as after precontraction with 50 mM KCl. Relaxations to nitroprusside were also studied in noradrenaline-precontracted rings in the presence of 1 mM tetraethylammonium.

2.8. Data presentation and statistical analysis of results

The maximal contractions were expressed in grams and related to tissue dry weight (g/mg) and the EC_{50} for noradrenaline and KCl in each ring was calculated as a percentage of maximal response. The relaxations in response to acetylcholine, cromakalim, isoprenaline, and nitroprusside were presented as a percentage of pre-existing contractile force. The EC_{50} values for acetylcholine and nitroprusside were calculated as percentage of 1 μM noradrenaline-induced precontraction. The EC_{50} values were calculated with a computer program and presented as the negative logarithm (pD_2), which values were also used in the statistical analysis.

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. All results are expressed as means ± S.E.M. Differences were considered significant when $P < 0.05$.

2.9. Drugs

The following drugs were used: enalapril maleate, losartan potassium (Merck Pharmaceutical, Wilmington, DE, USA), acetylcholine chloride, cromakalim, isoprenaline hydrochloride, N^G-L- arginine methyl ester hydrochloride, tetraethylammonium (Sigma, St. Louis, MO, USA), L-noradrenaline L-hydrogentartrate and sodium nitroprusside (Fluka Chemie, Buchs SG, Switzerland), and diclofenac (Voltaren injection solution, Ciba-Geigy, Basel, Switzerland). Enalapril and losartan were dissolved directly in tap water. The stock solutions of the compounds used in the in vitro studies were dissolved in distilled water, with the exception of cromakalim (in 50% ethanol). All solutions were freshly prepared before use and protected from light.

3. Results

3.1. Blood pressure, heart rate, heart and body weights

The systolic blood pressure in untreated SHR increased during the 10-week long follow up, whereas no significant change was observed in WKY. Enalapril and losartan treatments beginning at the age of 7 weeks completely prevented the elevation of blood pressures in SHR (Fig. 1). Heart rate did not differ in SHR and WKY, but it was somewhat reduced by losartan and enalapril administrations in SHR. Cardiac hypertrophy was totally prevented in SHR by enalapril and losartan, relative heart weights of enalapril- and losartan-treated SHR not differing from those of WKY. The losartan and enalapril therapies did not affect body weight in either strain (Table 1).

3.2. Arterial relaxations

Since the relaxation responses of enalapril- and losartan-treated WKY rats did not show any differences from those of untreated WKY rats, the graphs of enalapril- and losartan treated WKY rats were omitted from Figs. 2–5 for clarity reasons.

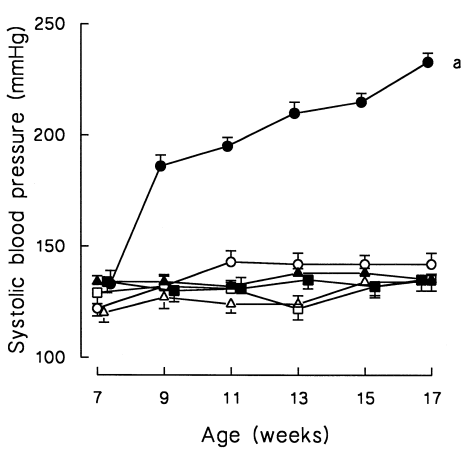

Fig. 1. Systolic blood pressures in untreated spontaneously hypertensive rats (SHR, ●), losartan-treated SHR (■), enalapril-treated SHR (▲), untreated Wistar–Kyoto rats (WKY, ○), losartan-treated WKY (□), and enalapril-treated WKY (△). Symbols indicate mean ± S.E.M., $n = 10–15$ in each group, $^aP < 0.05$ SHR vs. all other groups, ANOVA for repeated measurements.

Table 1
Experimental group data at close of the study

	SHR	LSHR	ESHR	WKY	LWKY	EWKY
Body weight (g)	305 ± 3	295 ± 5	294 ± 5	303 ± 2	305 ± 11	311 ± 6
Heart weight (mg)	1163 ± 410	780 ± 34[a]	780 ± 44[a]	859 ± 42[a]	708 ± 28[a,b]	720 ± 42[a,b]
Heart/body weight (mg/g)	3.83 ± 0.15	2.64 ± 0.11[a]	2.66 ± 0.15[a]	2.84 ± 0.14[a]	2.34 ± 0.12[a,b]	2.32 ± 0.15[a,b]
Heart rate (beats/min)	349 ± 7	326 ± 4[a]	326 ± 7[a]	338 ± 6	331 ± 3	336 ± 7

Values are means ± S.E.M., $n = 10–11$ for all groups.
SHR, LSHR and ESHR, untreated, losartan-treated and enalapril-treated spontaneously hypertensive rats, respectively; WKY, LWKY and EWKY, untreated, losartan-treated and enalapril-treated Wistar–Kyoto rats, respectively.
[a] $P < 0.05$ when compared with the SHR group; [b] $P < 0.05$ vs. WKY (Bonferroni test).

3.2.1. Endothelium-dependent relaxations

The relaxations induced by acetylcholine in noradrenaline (1 μM)-precontracted mesenteric arterial rings were impaired in untreated SHR when compared with WKY. These responses were clearly improved in SHR by the enalapril- and losartan-treatments, the relaxations not differing from those of WKY. Cyclooxygenase inhibition with diclofenac (3 μM) improved relaxation to acetylcholine in untreated SHR ($P < 0.002$), but not in the other groups. The NO synthase inhibitor, L-NAME (0.1 mM; in the presence of diclofenac), diminished the relaxations of noradrenaline-precontracted rings to acetylcholine in all

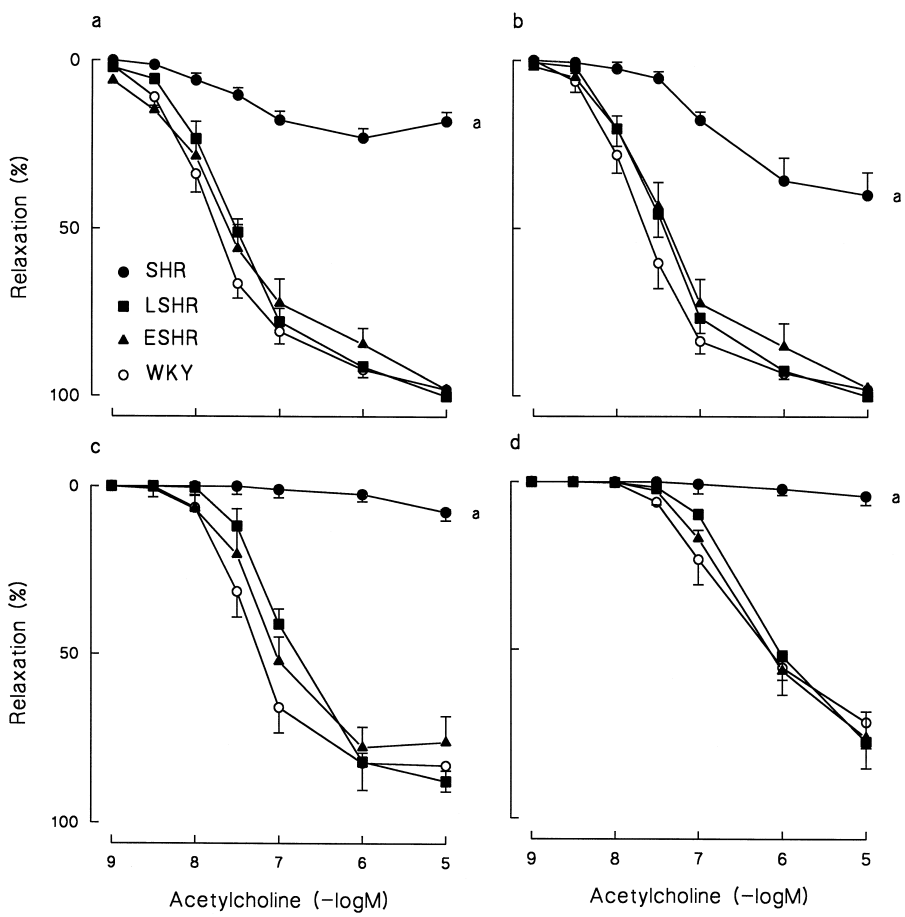

Fig. 2. Relaxation responses to acetylcholine after precontraction with 1 μM noradrenaline in endothelium-intact mesenteric arterial rings of SHR, LSHR, ESHR and WKY, untreated, losartan-treated, enalapril-treated spontaneously hypertensive rats, and untreated Wistar–Kyoto rats, respectively (a). The relaxations were also induced in the presence of 3 μM diclofenac (b); diclofenac and 0.1 mM L-NAME (c); diclofenac, L-NAME and 1 mM tetraethylammonium (c). Symbols indicate means ± S.E.M., $n = 8–13$ in each group, [a] $P < 0.05$ SHR vs. all other groups, ANOVA for repeated measurements.

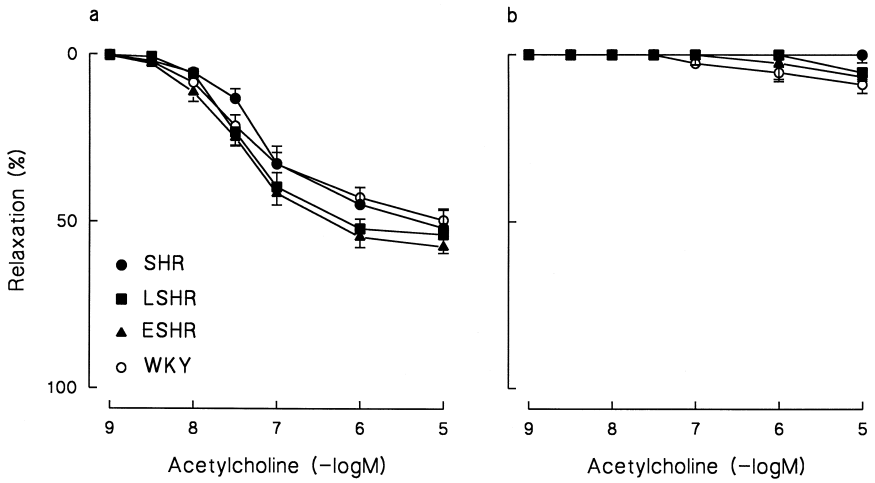

Fig. 3. Relaxation responses to acetylcholine after precontraction with 50 mM potassium chloride in endothelium-intact mesenteric arterial rings of SHR, LSHR, ESHR and WKY, untreated, losartan-treated, enalapril-treated spontaneously hypertensive rats, and untreated Wistar–Kyoto rats, respectively (a). The relaxations were also induced in the presence of 3 μM diclofenac and 0.1 mM L-NAME (b). Symbols indicate means ± S.E.M., $n = 8–13$ in each group.

groups ($P < 0.03$), and completely abolished the response in untreated SHR. Tetraethylammonium (1 mM), which is a rather selective blocker of large conductance Ca^{2+}-activated K^+ channels at the concentration used in this study (Kitazono et al., 1995), induced a further reduction in the remaining relaxations to acetylcholine in the study groups ($P < 0.001$) (Fig. 2; Table 2). The relaxations to acetylcholine during precontraction with KCl (50 mM), i.e., under conditions preventing hyperpolarization (Adeagbo and Triggle, 1993), were comparable in all six study groups. In addition, the responses to acetylcholine in KCl-precontracted rings were practically abolished in the presence of diclofenac and L-NAME in all groups (Fig. 3).

3.2.2. Endothelium-independent relaxations

The relaxations of noradrenaline-precontracted rings to the cromakalim, isoprenaline and nitroprusside, three different vasodilators acting via opening of ATP-sensitive K^+ channels (K_{ATP}), activation of β-adrenoceptors and the formation of exogenous NO, respectively, were impaired in untreated SHR when compared with the WKY groups. Losartan and enalapril therapies markedly enhanced these responses, the relaxations to cromakalim and nitroprusside not differing from those of normotensive controls, while the responses to isoprenaline were still somewhat impaired in losartan-treated SHR when compared with WKY. Tetraethylammonium diminished nitroprusside-induced relax-

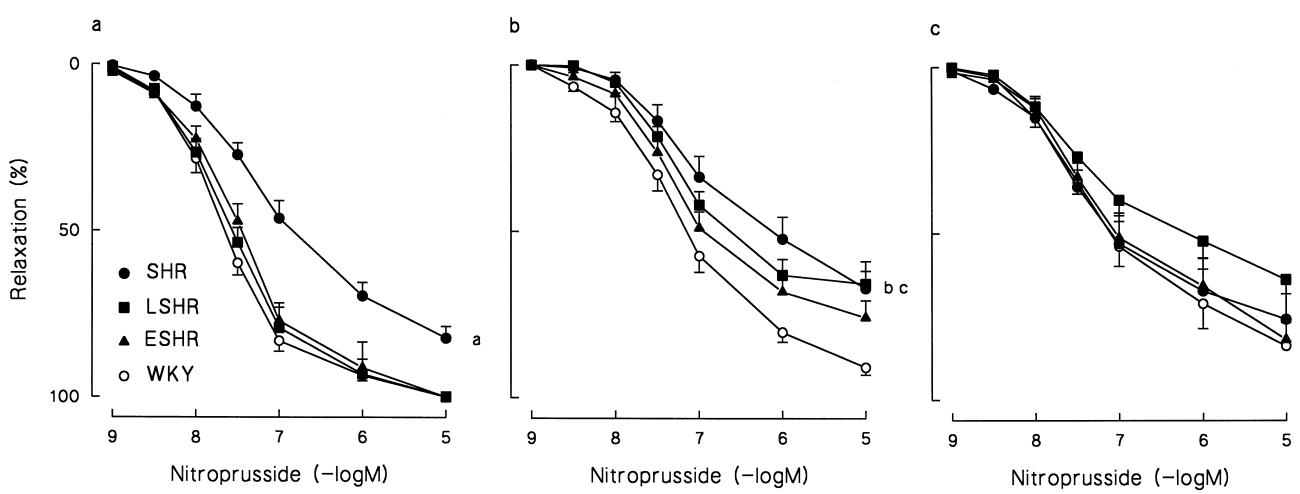

Fig. 4. Relaxation responses to nitroprusside after precontraction with 1 μM noradrenaline (a, b) and with 50 mM potassium chloride (c) in endothelium-denuded mesenteric arterial rings of SHR, LSHR, ESHR and WKY, untreated, losartan-treated, enalapril-treated spontaneously hypertensive rats, and untreated Wistar–Kyoto rats, respectively. The relaxations were also induced in the presence of 1 mM tetraethylammonium (b). Symbols indicate means ± S.E.M., $n = 9–12$ in each group, $^{a}P < 0.05$ SHR vs. all other groups, $^{b}P < 0.05$ SHR vs. WKY, $^{c}P < 0.05$ LSHR vs. WKY, ANOVA for repeated measurements.

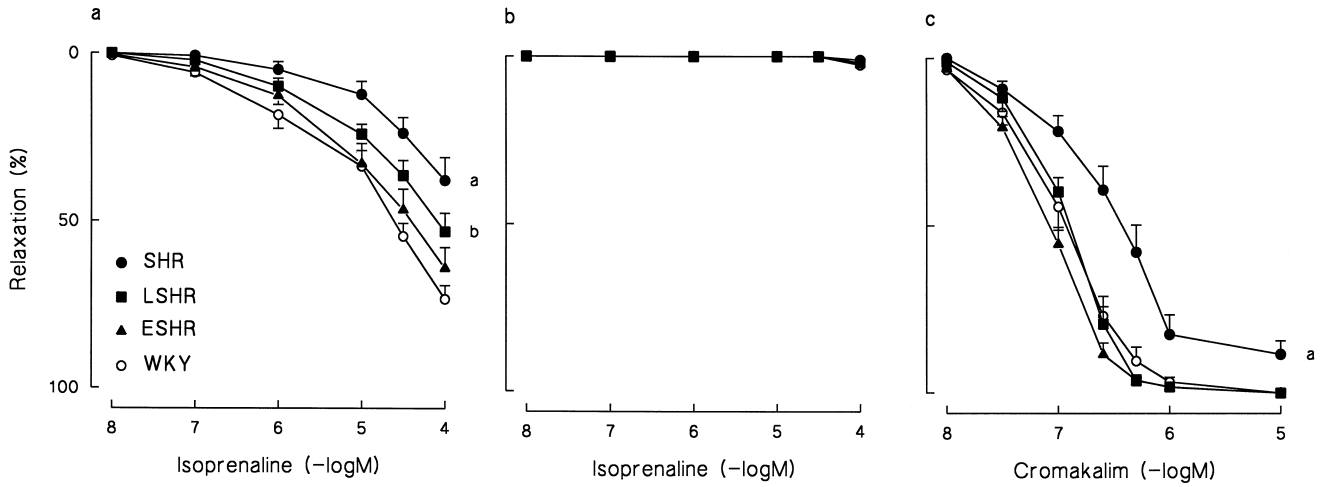

Fig. 5. Relaxation responses to isoprenaline after precontraction with 1 μM noradrenaline (a) and after precontraction with 50 mM potassium chloride (b), and relaxation responses to cromakalim after precontraction with 1 μM noradrenaline (c) in endothelium-denuded mesenteric arterial rings of SHR, LSHR, ESHR, and WKY untreated, losartan-treated, enalapril-treated spontaneously hypertensive rats, and untreated Wistar–Kyoto rats, respectively. Symbols indicate means ± S.E.M., $n = 8–13$ in each group, [a]$P < 0.05$ SHR vs. all other groups, [b]$P < 0.05$ LSHR vs. WKY, ANOVA for repeated measurements.

ations in all groups ($P < 0.05$), and abolished the difference between both drug-treated and the untreated SHR groups. When hyperpolarization of smooth muscle was prevented by precontracting the preparations with KCl, no differences were found in relaxations to nitroprusside between any of the study groups, and the dilatations to isoprenaline and cromakalim were completely abolished (Figs. 4 and 5; Table 2; the response to cromakalim after KCl-precontraction not shown).

3.3. Receptor- and depolarization-mediated contractions

Maximal contractile force generation to noradrenaline in the absence and presence of L-NAME and diclofenac was attenuated in endothelium-intact rings of untreated SHR when compared with WKY, although the sensitivity (i.e., pD_2 values) to noradrenaline was comparable in these groups. In contrast, the maximal contractions to noradrenaline in losartan and enalapril-treated SHR (both in the absence and presence of L-NAME and diclofenac) did not differ from those of WKY. There were no significant differences in the sensitivity to noradrenaline between control SHR and losartan- and enalapril-treated SHR, while in the presence of diclofenac and L-NAME the sensitivity was somewhat lower in enalapril-SHR when compared with the untreated controls. Furthermore, enalapril-SHR showed lower sensitivity to noradrenaline than losartan-SHR both in the absence and presence of L-NAME and diclofenac (Table 3). Maximal contractile force generation of endothelium-denuded arterial rings to KCl was compa-

Table 2
The negative logarithm of the concentration producing 50% relaxation of isolated mesenteric arterial rings

	SHR	LSHR	ESHR	WKY	LWKY	EWKY
Relaxation to acetylcholine after precontraction with noradrenaline	–	7.50 ± 0.09	7.51 ± 0.22	7.73 ± 0.09	7.70 ± 0.08	7.57 ± 0.18
with diclofenac	–	7.47 ± 0.12	7.29 ± 0.25	7.65 ± 0.10	7.47 ± 0.11	7.46 ± 0.11
with diclofenac and L-NAME	–	6.83 ± 0.13[b]	7.07 ± 0.18	7.31 ± 0.10	7.02 ± 0.11	6.71 ± 0.18
with diclofenac, L-NAME and tetraethylammonium	–	6.04 ± 0.16	6.23 ± 0.20	6.42 ± 0.18	6.29 ± 0.17	6.56 ± 0.10
Relaxation to nitroprusside after precontraction with noradrenaline (E-)	6.81 ± 0.17	7.55 ± 0.07[a]	7.45 ± 0.07[a]	7.65 ± 0.06[a]	7.56 ± 0.11[a]	7.62 ± 0.08[a]
with tetraethylammonium	6.33 ± 0.27	6.51 ± 0.19	6.65 ± 0.26	7.12 ± 0.11[a]	6.98 ± 0.10	6.95 ± 0.14
Relaxation to nitroprusside after precontraction with potassium chloride (E-)	6.92 ± 0.26	6.98 ± 0.23	7.04 ± 0.16	7.17 ± 0.11	7.14 ± 0.19	6.81 ± 0.25

Values are means ± S.E.M., $n = 8–13$ for all groups.
SHR, LSHR and ESHR, untreated, losartan-treated and enalapril-treated spontaneously hypertensive rats, respectively; WKY, LWKY and EWKY, untreated, losartan-treated and enalapril-treated Wistar–Kyoto rats, respectively.
L-NAME, N^G-nitro-L-arginine methyl ester; E-, endothelium-denuded arterial rings; –, the relaxations did not reach the level of 50%.
[a]$P < 0.05$ when compared with the SHR group; [b]$P < 0.05$ vs. WKY (Bonferroni test).

Table 3
Parameters of contractile responses of isolated mesenteric arterial rings in the experimental groups

	SHR	LSHR	ESHR	WKY	LWKY	EWKY
Contraction to noradrenaline						
pD_2	6.25 ± 0.09	6.54 ± 0.13	6.09 ± 0.06[c]	6.34 ± 0.09	6.38 ± 0.15	6.13 ± 0.13
pD_2 with L-NAME and diclofenac	6.44 ± 0.09	6.65 ± 0.17	6.12 ± 0.05[a,b,c]	6.39 ± 0.08	6.50 ± 0.12	6.18 ± 0.17
Maximal force (g/mg)	3.08 ± 0.25	5.04 ± 0.70[a]	6.39 ± 0.52[a]	5.81 ± 0.64[a]	6.81 ± 0.83[a]	5.76 ± 0.99[a]
Maximal force with L-NAME and diclofenac (g/mg)	3.34 ± 0.29	4.51 ± 0.74	6.56 ± 0.58[a,c]	5.89 ± 0.68[a]	7.52 ± 0.77[a]	6.13 ± 0.86[a]
Contraction to potassium chloride (E-)						
pD_2	1.63 ± 0.01	1.61 ± 0.02	1.59 ± 0.02	1.56 ± 0.02[a]	1.50 ± 0.02[a,b,c]	1.55 ± 0.03[a]
Maximal force (g/mg)	4.02 ± 0.49	5.83 ± 0.88	4.66 ± 0.49	4.22 ± 0.66	4.52 ± 0.67	5.39 ± 0.87

Values are means ± S.E.M., $n = 8–13$ for all groups.
SHR, LSHR and ESHR, untreated, losartan-treated and enalapril-treated spontaneously hypertensive rats, respectively; WKY, LWKY and EWKY, untreated, losartan-treated and enalapril-treated Wistar–Kyoto rats, respectively.
L-NAME, N^G-nitro-L-arginine methyl ester; E-, endothelium-denuded arterial rings; pD_2 is the negative logarithm of the concentration of agonist producing 50% of maximal contractile response.
[a] $P < 0.05$ when compared with the SHR group; [b] $P < 0.05$ vs. WKY; [c] $P < 0.05$ vs. LSHR (Bonferroni test).

rable in all study groups, and all WKY groups showed lower sensitivity to KCl than untreated SHR. Losartan but not enalapril further diminished the sensitivity to KCl in WKY, while in SHR the treatments were without effect on the sensitivity to KCl (Table 3).

4. Discussion

The present study showed that treatment with either losartan or enalapril completely prevented the development of hypertension and the associated cardiac hypertrophy in SHR. These results agree with previous investigations (Oddie et al., 1993; Schoemaker et al., 1994), which have stressed the importance of the renin–angiotensin system in the pathogenesis of high blood pressure in this model of genetic hypertension.

Acetylcholine relaxes arterial smooth muscle by releasing several dilatory factors from the vascular endothelium; the major contributors being NO, prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) (Busse and Fleming, 1993). In the present study, the relaxations to acetylcholine in noradrenaline-precontracted rings were attenuated in SHR, whereas these responses were completely normalized by losartan and enalapril, in accordance with previous findings (Tschudi et al., 1994). Since both of these therapies comparably augmented endothelium-dependent relaxations, this beneficial effect on the vasculature was probably mediated via the reduction of blood pressure and inhibition of the actions of angiotensin II.

One explanation to the attenuated relaxations in hypertension is enhanced release of endothelium-derived contracting factors (Lüscher and Vanhoutte, 1986). Previously, endothelium-dependent vasoconstrictor responses have been shown to be blocked by cyclooxygenase inhibition in SHR (Takase et al., 1994). In the present study, the cyclooxygenase inhibitor diclofenac enhanced the relaxations to acetylcholine only in untreated SHR. This suggests an imbalance in the production of vasoconstrictor and vasodilator prostanoids in the vessels of untreated SHR which favours vasoconstriction. Since diclofenac had no effect on the response to acetylcholine in the other groups, the present antihypertensive therapies appeared to correct this imbalance of the cyclooxygenase pathway in SHR.

Angiotensin converting enzyme inhibition has been suggested to potentiate endothelium-dependent dilatation in the aorta of normotensive and hypertensive animals by enhancing the availability of NO (Gohlke et al., 1993; Berkenboom et al., 1995). In addition, angiotensin II antagonism and angiotensin converting enzyme inhibition have been found to comparably improve endothelial function in the aorta and coronary arteries of SHR, the underlying mechanism possibly being increased availability of NO (Tschudi et al., 1994; Rodrigo et al., 1997). In the present study, however, the mesenteric arteries of losartan- and enalapril-treated SHR and all WKY groups showed distinct NO synthase inhibitor-resistant relaxations to acetylcholine, suggesting that endothelial products other than NO were mediating the enhanced endothelium-dependent vasodilatations.

Recent investigations have indicated that endothelium-mediated relaxations which remain resistant to both NO synthase and cyclooxygenase inhibitions are mediated by EDHF (Cohen and Vanhoutte, 1995). The chemical characteristics of EDHF remain unknown, but functionally this factor is a K^+ channel opener (Cohen and Vanhoutte, 1995). The action of EDHF can be eliminated by membrane depolarization with high concentrations of KCl, and under these conditions the relaxation to acetylcholine thus mainly reflects the effects of NO (Adeagbo and Triggle, 1993). In contrast, during receptor-mediated precontractions EDHF remains operative (Adeagbo and Triggle,

1993). In this study, losartan and enalapril treatments enhanced acetylcholine-induced relaxations in noradrenaline-precontracted preparations, but these treatments did not alter acetylcholine-induced relaxations in KCl-precontracted preparations. This suggests that the improvement of endothelium-dependent relaxation following long-term angiotensin II antagonism and angiotensin converting enzyme inhibition was largely mediated via hyperpolarization mechanisms.

The action of EDHF can also be inhibited by blockers of K^+ channels (Cohen and Vanhoutte, 1995). In rat mesenteric artery, apamin (inhibitor of small conductance Ca^{2+}-activated K^+ channels) has been found to reduce the L-NAME insensitive relaxations to acetylcholine by 55%, and to completely abolish these relaxations when combined with charybdotoxin (inhibitor of large conductance Ca^{2+}-activated K^+ channels) (Waldron and Garland, 1994). In the present study, tetraethylammonium (inhibitor of large conductance Ca^{2+}-activated K^+ channels) partially inhibited the diclofenac and L-NAME-insensitive relaxation to acetylcholine in WKY, as well as in losartan- and enalapril-treated SHR, thus providing further evidence that the improved endothelium-dependent relaxation following these therapies was mediated via the activation of K^+ channels and subsequent hyperpolarization of smooth muscle.

The endothelium-independent dilatations induced by isoprenaline, nitroprusside and cromakalim were also attenuated in untreated SHR. Losartan and enalapril therapies normalized the responses to nitroprusside and cromakalim, and improved vasodilatation to isoprenaline. This enhancement of vasodilatation via exogenous NO, opening of K^+ channels and activation of β-adrenoceptors suggests that improvement of general vascular relaxation properties (e.g., regulation of intracellular Ca^{2+}) may also have played a role in the augmented endothelium-dependent relaxations in losartan- and enalapril-treated SHR. Moreover, exogenous NO has been shown to hyperpolarize smooth muscle of guinea pig uterine artery and rat mesenteric artery (Tare et al., 1990; Garland and Mcpherson, 1992), while charybdotoxin and tetraethylammonium have been shown to decrease relaxation to NO in guinea pig pulmonary artery (Bialecki and Stinson-Fisher, 1995). In addition, NO has been proposed to directly activate Ca^{2+}-activated K^+ channels (K_{Ca}) in vascular smooth muscle (Bolotina et al., 1994). Isoprenaline has also been reported to open K_{ATP} in the smooth muscle of rat mesenteric artery (Randall and McCulloch, 1995), activate K_{Ca} in the smooth muscle of guinea pig basilar artery (Song and Simard, 1995), and cause endothelium-independent hyperpolarization of smooth muscle in pig coronary artery (Beny and Pacicca, 1994). Thus, improved function of K^+ channels in the smooth muscle of losartan- and enalapril-treated SHR could well explain the enhanced endothelium-independent relaxations, as well as the augmented endothelium-dependent relaxations, which appeared to be mediated via hyperpolarization mechanisms. This view is supported by the fact that, in this study, the relaxations to isoprenaline were absent, and the differences in relaxations to nitroprusside were abolished when the responses were induced after KCl-precontraction, i.e., under conditions preventing hyperpolarization. Furthermore, the addition of tetraethylammonium abolished the differences between the treated and untreated SHR in relaxations to nitroprusside during noradrenaline-precontraction, supporting the view that K^+ channels in smooth muscle were mediating the enhanced dilatory responses to nitroprusside in treated SHR. The finding that the relaxations of noradrenaline-precontracted rings to K^+ channel opener cromakalim were markedly improved after the therapies in SHR further supports the concept of improved K^+ channel function following losartan and enalapril therapies in SHR. Moreover, long-term therapy with the vasodilator hydralazine has recently been found to correct the impaired action of levcromakalim on ATP-sensitive K^+ channels in SHR (Ohya et al., 1996), suggesting that antihypertensive treatment favourably affects the function of smooth muscle cell membrane in hypertension.

Various approaches can be applied to investigate the possible differences in vasoconstrictor responses between hypertensive and normotensive blood vessels. The arterial contractile forces can be related to segment length, segment weight, medial cross-sectional area, or lumen diameter, and thus the findings depend on the experimental setting which has been applied (Mulvany et al., 1991; Arvola et al., 1993; Bennett et al., 1996). Moreover, a normotensive control group must be included in the experiments which elucidate the effects of antihypertensive therapy on the control of arterial tone in hypertension, since otherwise, it is difficult to know whether the observed change is a beneficial one or not. In the present study, losartan and enalapril treatments increased the maximal contractile force generated by noradrenaline in artery preparations from SHR, but the responses were not different from those in arterial preparations from untreated WKY. Since the normalization of the maximal response to noradrenaline by chronic angiotensin converting enzyme inhibition has also been reported in the mesenteric resistance arteries of SHR (Bennett et al., 1996), long-term inhibition of the renin–angiotensin system appears to normalize the noradrenaline-induced vasoconstrictor responses in SHR. The contractile sensitivity to KCl was higher in SHR when compared with WKY whereas the maximal contractile force was comparable, and neither of the therapies affected the responses to KCl in SHR. In agreement with this finding, maximal contractions to KCl have been found to remain unaffected by both angiotensin II antagonist and angiotensin converting enzyme inhibitor treatment in the aorta of SHR (Rodrigo et al., 1997). Thus, the present results suggest that both losartan and enalapril therapies restored the impaired receptor-mediated contractions to noradrenaline in the mesenteric artery of SHR

without affecting the sensitivity of smooth muscle to depolarization by KCl.

In conclusion, losartan and enalapril therapies prevented the development of hypertension in SHR, an effect which was associated with improved endothelium-dependent and -independent arterial relaxation. The endothelium-mediated relaxation in enalapril- and losartan-treated hypertensive rats was augmented in the absence and presence of NO synthase inhibition but not under conditions preventing hyperpolarization, and endothelium-independent relaxation was enhanced during receptor- but not during depolarization-mediated precontractions. Therefore, these results for the first time suggest that improved vasodilatation of mesenteric arterial rings following losartan and enalapril therapies could be attributed to enhanced hyperpolarization of arterial smooth muscle in this model of experimental hypertension.

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