Endothelial function in spontaneously hypertensive rats: influence of quinapril treatment

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1 Angiotensin converting enzyme (ACE) inhibition has been shown to restore the impaired endothelial function in hypertension, but the mediators underlying the promoted endothelium-dependent dilatation have not been fully characterized. Therefore, we investigated the effects of 10-week-long quinapril therapy (10 mg kg⁻¹ day⁻¹) on responses of mesenteric arterial rings *in vitro* from spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats.

2 Endothelium-dependent relaxations of noradrenaline (NA)-precontracted rings to acetylcholine (ACh) and adenosine 5'-diphosphate (ADP) were similar in WKY rats and quinapril-treated SHR and more pronounced than in untreated SHR. The nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) attenuated the relaxations in both WKY groups and quinapril-treated SHR, and completely inhibited them in untreated SHR. When endothelium-dependent hyperpolarization was prevented by precontraction of the preparations with potassium chloride (KCl), no differences were found in relaxations to ACh and ADP between the study groups. In addition, in NA-precontracted rings the L-NAME- and indomethacin-resistant relaxations to ACh were partially prevented by apamin, an inhibitor of calcium-activated potassium channels.

3 Interestingly, in quinapril-treated SHR but not in the other groups, exogenous bradykinin potentiated the relaxations to ACh in both NA- and KCl-precontracted arterial rings.

4 Contractile sensitivity of endothelium-intact rings to NA was reduced in SHR by quinapril, and was more effectively increased by L-NAME in quinapril-treated than untreated SHR.

5 In conclusion, since the relaxations to ACh and ADP in quinapril-treated SHR were augmented in the absence and presence of NO synthesis inhibition but not under conditions which prevented hyperpolarization, enhanced endothelium-dependent relaxation after long-term ACE inhibition can be attributed to increased endothelium-dependent hyperpolarization. However, the potentiation of the response to ACh by exogenous bradykinin in quinapril-treated SHR, as well as the increased attenuating effect of the endothelium on NA-induced contractions in these animals appear to result from enhanced endothelium-derived NO release.

Keywords: Angiotensin-converting enzyme inhibition; arterial smooth muscle; blood pressure; bradykinin; endothelium; quinapril; spontaneously hypertensive rat; Wistar-Kyoto rat

Introduction

Vascular endothelium plays an important role in the regulation of arterial tone by producing dilator mediators which include endothelium-derived relaxing factor (EDRF; probably identical with nitric oxide (NO)), prostacyclin (PGI₂) and endothelium-derived hyperpolarizing factor (EDHF) (Chen *et al.*, 1988; Moncada *et al.*, 1991; Fujii *et al.*, 1992). Several reports have shown endothelial function to be impaired in hypertension: endothelium-dependent relaxations are diminished in spontaneously hypertensive rats (SHR) (Watt & Thurston, 1989; Clozel *et al.*, 1990; Arvola *et al.*, 1993; Rubanyi *et al.*, 1993; Kähönen *et al.*, 1994) as well as in hypertensive patients (Treasure *et al.*, 1992), and reduced NO production has been suggested to underlie the attenuated endothelium-mediated dilatations (Tsefamiariam & Halpern, 1988; Clozel *et al.*, 1990). However, deficient endothelium-dependent hyperpolarization has recently been shown to account for the impaired relaxation to acetylcholine (ACh) in SHR mesenteric arteries, while the role of NO in the response was well preserved, and the participation of PGI₂ in the relaxation appeared to be marginal (Fujii *et al.*, 1992; 1993; Kähönen *et al.*, 1994; Li *et al.*, 1994). Furthermore, ACh is known to evoke contractions in blood vessels of SHR but not of Wistar-Kyoto (WKY) rats via the release of endothelium-derived contractile

factors (EDCF). Thus, the decreased relaxant effect of ACh in SHR may not result from deficient dilator autacoid production alone, but also from simultaneous release of EDCF from the endothelium (Lüscher & Vanhoutte, 1986; Jameson *et al.*, 1993; Takase *et al.*, 1994).

The antihypertensive action of angiotensin converting enzyme (ACE) inhibitors is primarily based on the inhibition of angiotensin II formation. The vascular wall appears to have a renin-angiotensin system of its own which ACE inhibitors directly inhibit (Levy *et al.*, 1990), even more effectively than ACE circulating in the blood stream (Baudin & Drouet, 1989). Long-term ACE inhibitor therapy has been shown to augment endothelium-dependent relaxation responses of arteries in SHR (Clozel *et al.*, 1990; Arvola *et al.*, 1993; Gohlke *et al.*, 1993; Rubanyi *et al.*, 1993) and in normotensive animals (Bossaller *et al.*, 1992), and to enhance ACh-induced vasodilatation *in vivo* in normotensive human subjects (Nakamura *et al.*, 1992). In addition to reduced angiotensin II generation, ACE inhibitors diminish the degradation of bradykinin, which in turn stimulates the synthesis of NO and PGI₂ in endothelial cells (Wiemer *et al.*, 1991) and also promotes endothelium-dependent hyperpolarization *in vitro* (Illiano *et al.*, 1994). However, whether the favourable long-term influences of ACE inhibition on endothelial function can be attributed to enhanced NO or PGI₂ generation, promoted endothelium-dependent hyperpolarization, or reduced contractile factor release from the endothelium is not fully understood.

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Recently, we have reported that ACE inhibitor therapy improved both endothelium-dependent and -independent arterial relaxation in SHR (Arvola *et al.*, 1993). The present study was designed to examine in detail the effects of long-term treatment with quinapril, an orally active non-sulphydryl compound (Kaplan *et al.*, 1984), on vascular responses in SHR and WKY rats. Special attention was paid to the evaluation of the roles of NO, PGI₂, EDHF and EDCF in endothelium-mediated responses. Since acute ACE inhibition is known to potentiate the effects of kinins in endothelial cells (Wiemer *et al.*, 1991; Illiano *et al.*, 1994), the dilator responses to bradykinin were also examined after the chronic quinapril therapy.

Methods

Animals and experimental design

Male SHR (Okamoto-Aoki strain) and age-matched WKY rats were obtained from Møllegaard's Breeding Centre, Ejby, Denmark. The animals were housed four to a cage in a standard experimental animal laboratory (illuminated 06 h 00 min–18 h 00 min, temperature +22°C), and had free access to drinking fluid (tap water) and food pellets (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 Inc., Woodland Hills, Ca., U.S.A.). At 7 weeks of age both SHR and WKY rats were divided into two groups of equal mean systolic blood pressures. Thereafter, SHR ($n=15$) and WKY rats ($n=15$) were given quinapril in drinking water in light-proof bottles, (average dose 10 mg kg⁻¹, fresh drug solutions being prepared daily), while untreated SHR ($n=15$) and normotensive WKY rats ($n=15$) were kept on normal drinking fluid. Quinapril therapy and weekly indirect blood pressure measurements continued for 10 more weeks until the animals were 17 weeks old. Thereafter quinapril administration was withdrawn 1 day before the rats were decapitated and exsanguinated. The hearts were removed and weighed, and the superior mesenteric arteries carefully excised and cleaned of adherent connective tissue. The experimental design of the study was approved by the Animal Experimentation Committee of the University of Tampere, Finland.

Mesenteric arterial responses in vitro

Five successive standard sections (3 mm in length) of the mesenteric artery from each animal were cut, beginning 1 cm distally from the mesenteric artery-aorta junction. In the four most distal rings the endothelium was left intact and from the first piece, vascular endothelium was removed by gently rubbing with a jagged injection needle (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, NaHCO₃ 25.0, glucose 11.1, CaCl₂ 1.6, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, and aerated with 95% O₂ and 5% CO₂. The rings were initially equilibrated for 1 h at +37°C at a resting force 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 Co., Quincy, Ma., U.S.A.). The presence of intact endothelium in vascular preparations was confirmed by an almost complete relaxation response (>75%) to 1 μ M ACh in 1 μ M noradrenaline (NA)-precontracted rings, and the absence of endothelium by the lack of this relaxation response. If any relaxation was seen in endothelium-denuded rings, the endothelium was further rubbed.

Vascular preparation 1 After 30 min, the relaxation responses of endothelium-denuded preparations to 3-morpholinoydno-

nimine (SIN-1) were determined. The relaxation responses were elicited after full precontraction with 1 μ M NA, which resulted in approximately 60% of the maximal contractile response attained in each group. The next concentration of SIN-1 was added only after the previous level of relaxation was stable.

Vascular preparation 2 After a 30 min stabilization period, concentration-response curves for NA were determined cumulatively in endothelium-intact rings. Thereafter relaxation responses to ACh were examined in rings precontracted with 1 μ M NA and 30 min later with 60 mM KCl. Then the three responses were repeated in the presence of 0.1 mM N^G-nitro-L-arginine methyl ester (L-NAME) with a 30 min stabilization period between each trial.

Vascular preparation 3 Relaxation responses to ACh were examined in endothelium-intact mesenteric arterial rings precontracted with 1 μ M NA. The responses to ACh were also elicited in the presence of 10 μ M indomethacin; in the presence of indomethacin and 0.1 mM L-NAME; and in the presence of indomethacin, L-NAME and 1 μ M apamin. The rings were allowed a 30 min equilibration period in PSS (containing the above agents) between each relaxant response.

Vascular preparation 4 The fourth endothelium-intact section of each mesenteric artery was used to study relaxation responses to adenosine 5'-diphosphate (ADP) in rings precontracted with 1 μ M NA and 30 min later with 60 mM KCl. Then the relaxation responses were also examined in the presence of 0.1 mM L-NAME. The rings were allowed a 30 min equilibration period in PSS between each response.

Vascular preparation 5 The most distal endothelium-intact ring of each mesenteric artery was used to study responses to bradykinin and 30 min later to ACh in the presence of 0.1 μ M bradykinin. The responses were generated cumulatively after full precontraction with 1 μ M NA and also after precontraction with 60 mM KCl. Cumulative relaxations to ACh in the presence of bradykinin were elicited only after the transient response (if observed) to bradykinin was over. The protocol was then repeated in the presence of 0.1 mM L-NAME. The rings were allowed a 30 min equilibration period between each response.

The NA-induced contractile responses were expressed in g and as percentages of the maximal response, and the pD₂₅ for NA in each ring was calculated as a percentage of the maximal response. The EC₂₅ values were calculated with a computer

Figure 1 Systolic blood pressures in untreated spontaneously hypertensive rats (SHR, □), quinapril-treated SHR (■, 10 mg kg⁻¹ day⁻¹), untreated Wistar-Kyoto (WKY, ○) rats and quinapril-treated WKY rats (●). Symbols indicate means with s.e. means, $n=10-12$ in each group; * $P<0.05$, ANOVA for repeated measurements.

programme and presented as the negative logarithm (pD_{25}): these values were also used in the statistical analysis. The EC_{25} for NA was calculated since the difference between the study groups was most pronounced at this point. The relaxations in response to ACh, ADP and SIN-1 were presented as a percentage of the pre-existing contractile force.

Drugs

The following drugs were used: quinapril hydrochloride (Goedecke AG, Freiburg, Germany), acetylcholine chloride, adenosine diphosphate, apamin, bradykinin, indomethacin, N^G -nitro-L-arginine methyl ester hydrochloride, (Sigma Chemical Co., St. Louis, Mo., U.S.A.), 3-morpholinodisodionimine (GEA Ltd., Copenhagen, Denmark) and (–)-noradrenaline L-hydrogentartrate (Fluka Chemie AG, Buchs SG, Switzerland).

Quinapril was 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 indomethacin (in 1.5 mM Na_2CO_3). All solutions were freshly prepared before use and protected from light.

Analysis of results

Statistical analysis was carried out by one-way analysis of variance (ANOVA) supported by the Bonferroni test in the case of pairwise between-group comparisons. When the data consisted of repeated observations at successive time points, ANOVA for repeated measurements was applied to investigate between-groups differences. All results are expressed as means with s.e.means. Differences were considered significant when $P < 0.05$.

Table 1 Experimental group data at close of the study

	SHR	Quin-SHR	WKY	Quin-WKY
Body weight (g)	312 ± 5	296 ± 6†	343 ± 5*	301 ± 7*†
Heart weight (mg)	1224 ± 33	911 ± 20*†	1030 ± 28*	888 ± 31*†
Heart-body weight ratio (mg g ⁻¹)	3.9 ± 0.1	3.1 ± 0.1*	3.0 ± 0.1*	3.1 ± 0.1*
Heart rate (beats min ⁻¹)	346 ± 10	327 ± 7	336 ± 6	339 ± 4

Values are mean ± s.e.mean, $n = 10-12$ for all groups. SHR and Quin-SHR, untreated and quinapril-treated spontaneously hypertensive rats, respectively; WKY and Quin-WKY, untreated and quinapril-treated Wistar-Kyoto rats, respectively. * $P < 0.05$ when compared with the SHR group; † $P < 0.05$ versus WKY (Bonferroni test).

Figure 2 Relaxations to acetylcholine (ACh) and adenosine diphosphate (ADP) in isolated endothelium-intact mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR, □), quinapril-treated SHR (■), untreated Wistar-Kyoto (WKY, ○) rats, and quinapril-treated WKY rats (●). The relaxations were induced after precontraction with 1 μ M noradrenaline in the absence (a and d) and presence (b and e) of 0.1 mM N^G -nitro-L-arginine methyl ester (L-NAME), and after precontraction with 60 mM KCl (c and f). Symbols indicate means with s.e.means, $n = 10-12$ in each group; * $P < 0.05$, ANOVA for repeated measurements. † $P < 0.05$ versus other groups (Bonferroni test).

Results

Blood pressure, heart weight and body weight

The systolic blood pressure of SHR was already higher at the beginning of the study than in WKY rats, and during the 10-week-long follow up it continued to increase in untreated SHR, whereas no significant change was observed in control WKY rats. Quinapril treatment beginning at the age of 7 weeks reduced blood pressure in both SHR and WKY rats below that of untreated WKY rats during the follow-up period (Figure 1). Cardiac hypertrophy was totally prevented in SHR by quinapril, relative heart weights of quinapril-treated SHR not differing from those of WKY rats (Table 1).

SHR and WKY rats on oral quinapril gained somewhat less weight than untreated SHR and WKY rats (Table 1). However, no signs of compromised well-being of the animals were observed by our experienced experimental animal laboratory staff. Chow intakes were comparable in all four study groups (data not shown).

Mesenteric arterial responses

The relaxations induced by ACh and ADP in endothelium-intact NA-precontracted ($1 \mu\text{M}$) mesenteric arterial rings were impaired in untreated SHR when compared with WKY rats. These responses were clearly improved in SHR by the quinapril treatment, the relaxations not differing from those of WKY rats. L-NAME (0.1 mM) diminished the relaxations of NA-precontracted rings effectively in both strains, the influence on ACh response being more pronounced in SHR than WKY rats, and comparable on ADP responses in the two rat strains. In quinapril-treated SHR the remaining relaxations in the presence of L-NAME were more pronounced ($30\text{--}100 \text{ nM}$ ACh; $1\text{--}100 \mu\text{M}$ ADP) than in the other groups (Figure 2). Apamin ($1 \mu\text{M}$) reduced the L-NAME and indomethacin-resistant relaxations to ACh by approximately $30\text{--}50\%$ in quinapril-treated SHR and in WKY rats (Figure 3). Interestingly, the relaxations to ACh and ADP during precontraction with KCl (60 mM), i.e. under conditions in which endothelium-derived hyperpolarization was prevented, were comparable in all

Figure 3 Relaxations of endothelium-intact mesenteric arterial rings after precontraction with $1 \mu\text{M}$ noradrenaline from untreated spontaneously hypertensive rats (SHR, $\square$), quinapril-treated SHR ($\blacksquare$), untreated Wistar-Kyoto (WKY, $\circ$) rats and quinapril-treated WKY rats ($\bullet$). The relaxation to acetylcholine consisted of an initial maximal response followed by a recontraction phase which is shown in the absence (a) and presence (b) of $10 \mu\text{M}$ indomethacin. The maximal relaxations to ACh in the presence of $10 \mu\text{M}$ indomethacin and 0.1 mM N^{G} -nitro-L-arginine methyl ester (L-NAME) (c) were partially inhibited by $1 \mu\text{M}$ apamin (d). Symbols indicate means with s.e.means, $n = 10\text{--}12$ in each group; $*P < 0.05$, ANOVA for repeated measurements.

four study groups (Figure 2). In addition, the responses to ACh and ADP in KCl-precontracted rings were practically abolished in the presence of L-NAME (0.1 mM) in all groups (maximal responses to ACh in the SHR, Quin-SHR, WKY and Quin-WKY groups were $-4.1 \pm 3.6\%$, $-3.5 \pm 1.9\%$, $-2.4 \pm 1.6\%$, $-2.2 \pm 1.0\%$, respectively).

Following maximal dilatations to ACh in NA-precontracted rings, the force of contraction rapidly increased in untreated SHR, while such recontractions were practically absent in the WKY groups and quinapril-treated SHR (Figure 3). The cyclo-oxygenase inhibitor, indomethacin (10 μM), slightly reduced the recontractions to ACh in untreated SHR, but had no effect on the responses in the other groups (Figure 3).

In NA-precontracted (1 μM) endothelium-intact mesenteric arterial rings, higher concentrations of bradykinin (0.1–1 μM) induced distinct but transient relaxations in quinapril-treated SHR, whereas only slight contractions were observed in WKY rats and untreated SHR (Figure 4). In the presence of 0.1 mM L-NAME the relaxations of quinapril-treated SHR to bradykinin, whether studied in NA- or KCl-precontracted rings, were totally abolished. Interestingly, when cumulative relaxations to ACh were induced in the presence of bradykinin (0.1 μM) in NA- and KCl-precontracted rings, the responses were enhanced in quinapril-treated SHR but not in the other groups. Again, L-NAME (0.1 mM) efficiently inhibited the relaxations in all study groups, the effect on the ACh response

in NA-precontracted rings being most marked in untreated SHR and lowest in quinapril-treated SHR (Figure 5). Furthermore, the response to ACh in the presence of bradykinin and L-NAME in quinapril-treated SHR appeared very similar to the response elicited without exogenous bradykinin under the same conditions (see Figure 2). In addition, in KCl-precontracted rings the relaxations to ACh in the presence of bradykinin were abolished by L-NAME in all groups (maximal responses to ACh in the SHR, Quin-SHR, WKY and Quin-WKY groups were $-1.7 \pm 3.8\%$, $-2.4 \pm 1.2\%$, $-2.7 \pm 1.6\%$, $-1.1 \pm 2.4\%$, respectively). No differences were found between the study groups in the relaxation responses induced by the NO donor SIN-1 (Figure 5).

The endothelium-intact vascular rings of untreated SHR and WKY rats showed comparable sensitivity (i.e. pD_{25} values) and maximal force generation to NA. Quinapril treatment reduced both sensitivity and maximal responses to NA in endothelium-intact rings of SHR. However, in the presence of L-NAME, and also in endothelium-denuded rings, the sensitivity to NA was corresponding in all groups, but maximal responses still remained diminished in quinapril-treated SHR. Thus, the increase in sensitivity to NA elicited by L-NAME was more pronounced in quinapril-treated than in untreated SHR (Table 2 and Figure 6).

Discussion

In the present study, the relaxations to ACh and ADP in NA-precontracted rings were impaired in SHR and were clearly enhanced by quinapril, the responses in the treated SHR not differing from those of WKY rats. Thus, these findings support the concept whereby long-term ACE inhibition favourably affects endothelial function in SHR (Clozel *et al.*, 1990; Arvola *et al.*, 1993; Rubanyi *et al.*, 1993). Since no significant differences were found in the relaxations to the NO donor, SIN-1, the sensitivity of arterial smooth muscle to NO was comparable in the study groups. Furthermore, inhibition of NO synthesis diminished relaxations of NA-precontracted rings to ACh and ADP more effectively in SHR than WKY rats, the responses being virtually absent in SHR in the presence of L-NAME. Hence, endothelium-mediated relaxations in SHR were largely mediated by NO, whereas the other groups showed distinct L-NAME resistant relaxations especially to ACh, these responses in quinapril-treated SHR being even more pronounced than in WKY rats. These results suggest that an endothelial product other than NO was responsible for enhanced responses to ACh and ADP in quinapril-treated SHR.

Interestingly, no significant differences between SHR and WKY rats were found in response to ACh and ADP when the relaxations were elicited in KCl-precontracted rings. Hence, in agreement with previous findings, endothelium-dependent relaxations in SHR are not impaired under all conditions (Li & Bukoski, 1993; Kähönen *et al.*, 1994). Several authors have reported that ACh can cause hyperpolarization of arterial smooth muscle *in vitro* (Chen *et al.*, 1988; Feletou & Vanhoutte, 1988; Bray & Quast, 1991; Garland & McPherson, 1992). Recent findings suggest that the hyperpolarizing effect of ACh is not mediated by NO (Garland & McPherson, 1992; Vanheel *et al.*, 1994), and the existence of EDHF has been proposed, although its exact identity still remains unknown (Chen *et al.*, 1988; Garland & McPherson, 1992; Fujii *et al.*, 1993; Li *et al.*, 1994). Nevertheless, membrane depolarization induced by precontracting the preparations with KCl eliminates the action of EDHF, and the remaining relaxation to ACh thus largely reflects the effect of NO, whereas under conditions of NA-induced precontraction, EDHF remains operative (Feletou & Vanhoutte, 1988; Adeagbo & Triggle, 1993). In the study described here, the responses to ACh and ADP were totally inhibited in KCl-precontracted preparations of all groups by L-NAME suggesting that EDHF and NO were responsible for the relaxations to these agonists. Since en-

Figure 4 Transient relaxations to bradykinin in quinapril-treated spontaneously hypertensive rats (SHR), depicted as a typical original tracing from 10 individual experiments (a). In (b) are shown maximal responses to bradykinin after precontraction with 1 μM noradrenaline in endothelium-intact mesenteric arterial rings from untreated SHR (solid columns), quinapril-treated SHR (stippled columns), untreated Wistar-Kyoto (WKY, open columns) rats and quinapril-treated WKY rats (hatched columns). Symbols indicate means with s.e. means, $n = 10$ –12 in each group; * $P < 0.05$, Bonferroni test.

dothelium-mediated responses were comparable in all groups during precontraction with KCl but were impaired in untreated SHR during precontraction with NA, and L-NAME also more efficiently diminished the responses in SHR, the impaired agonist-induced relaxations in hypertensive rats can be attributed to attenuated endothelium-dependent hyperpolarization, while the role of NO in the response appeared to be preserved. The fact that quinapril therapy of SHR did not affect relaxations in KCl-precontracted rings, while those induced in NA-precontracted rings were markedly enhanced, suggests that the hyperpolarization induced by ACh and ADP was especially augmented by quinapril. This conclusion is further supported by the finding that the relaxations in NA-precontracted rings in the presence of L-NAME were most marked in quinapril-treated SHR.

Waldron & Garland (1994) recently found that apamin, a blocker of Ca^{2+} -activated K^+ channels, reduced the L-NAME-insensitive relaxation by 55% in the rat mesenteric artery, and apamin together with charybdotoxin completely abolished

these responses. In the study reported here, apamin also partially inhibited the responses to ACh in WKY rats and quinapril-treated SHR, suggesting that these relaxations were indeed mediated by EDHF and the subsequent hyperpolarization of arterial smooth muscle.

ACh can induce endothelium-dependent contractions in arteries of SHR via the release of EDCF (Lüscher & Vanhoutte, 1986; Ito & Carretero, 1992; Auch-Schweik *et al.*, 1992b), whereas such responses are absent in WKY rats (Lüscher & Vanhoutte, 1986; Jameson *et al.*, 1993). The production of EDCF has been found to parallel closely the increase in blood pressure in SHR (Iwama *et al.*, 1992), and the contractile responses to ACh in arteries of these animals have been shown to be blocked by cyclo-oxygenase inhibition (Jameson *et al.*, 1993; Takase *et al.*, 1994). In the present study, maximal dilator responses to ACh were followed by rapid increases in contractile force in untreated SHR but not in WKY rats and quinapril-treated SHR. Furthermore, indomethacin slightly inhibited the reconstrictions in untreated SHR, sug-

Figure 5 Relaxations to acetylcholine in the presence of $0.1 \mu\text{M}$ bradykinin in endothelium-intact mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR, $\square$), quinapril-treated SHR ($\blacksquare$), untreated Wistar-Kyoto (WKY, $\circ$) rats and quinapril-treated WKY rats ($\bullet$). The relaxations were induced after precontraction with $1 \mu\text{M}$ noradrenaline in the absence (a) and presence (b) of 0.1 mM N^G -nitro-L-arginine methyl ester (L-NAME), and after precontraction with 60 mM KCl (c). Relaxations in endothelium-denuded rings were also induced by 3-morpholinysydnonimine (SIN-1) (d). Symbols indicate means with s.e. means, $n = 10-12$ in each group; $*P < 0.05$, ANOVA for repeated measurements.

gesting that constricting prostanoids were involved in these responses. However, indomethacin was without effect on the relaxation to ACh in the other groups, which suggests that products of the cyclo-oxygenase pathway were not playing a

significant role in the responses of WKY rats and quinapril-treated SHR. On the other hand, since quinapril therapy diminished the reconstrictions to ACh in SHR, the release of contractile factors from the endothelium was probably also reduced.

ACE inhibition *in vitro* diminishes the degradation of bradykinin, which in turn stimulates the synthesis of NO and PGI₂ in endothelial cells (Wiemer *et al.*, 1991). ACE inhibitors also potentiate the relaxations to bradykinin in several arteries (Auch-Schweik *et al.*, 1992a), an effect which involves the augmented release of both NO and EDHF (Mombouli *et al.*, 1992). Previously, in NA-precontracted rat mesenteric arteries, bradykinin has only been shown to induce vasoconstriction (Fasciolo *et al.*, 1990), which was also confirmed in the present study in WKY rats and untreated SHR. In contrast, in quinapril-treated SHR, higher concentrations of bradykinin induced relaxations in NA-precontracted rings, the explanation of which is not apparent from the present results. However, ACE inhibitors have recently been suggested to potentiate the actions of bradykinin at the level of the B₂-kinin receptor independently of the inhibition of ACE (Auch-Schweik *et al.*, 1993; Hecker *et al.*, 1994). According to the present results, such an interaction could have occurred in quinapril-treated SHR, or the long-term ACE inhibition by some other mechanism unmasked the dilator action of bradykinin. However, similar results were not observed in quinapril-treated WKY rats, which may indicate altered regulation of bradykinin receptors in SHR. Differences between these two strains in response to bradykinin have also been observed previously, since the coronary circulation in SHR has been reported to show significantly higher sensitivity to bradykinin paralleled by a higher release of NO when compared with WKY rats (Kelm *et al.*, 1992). The relaxations to bradykinin in quinapril-treated SHR were most probably elicited by NO since they were abolished by L-NAME.

Interestingly, bradykinin enhanced the relaxations to ACh in quinapril-treated SHR in the present study. In addition, the bradykinin-potentiated relaxations to ACh were effectively inhibited by L-NAME in both NA- and KCl-precontracted rings, and the responses to ACh in the presence of bradykinin and L-NAME appeared very similar to the responses elicited without exogenous bradykinin under the same conditions in all groups. Since bradykinin augmented the relaxation to ACh in both NA- and KCl-precontracted arterial rings, and this influence was effectively eliminated by NO synthesis inhibition, this potentiation appeared to result from enhanced endothelium-derived NO release following the long-term ACE inhibition. Taken together, bradykinin might also promote vasodilatation *in vivo* during ACE inhibitor therapy by potentiating the effects of other endothelium-mediated vasorelaxants. Additional investigations are clearly needed for the further characterization of this inhibition.

In the present study, maximal contractile force generation of endothelium-denuded arterial rings to NA was lower in

Figure 6 Concentration-response curves of endothelium-intact mesenteric arterial rings to noradrenaline in untreated spontaneously hypertensive rats (SHR, $\square$), quinapril-treated SHR (quin-SHR, $\blacksquare$), untreated Wistar-Kyoto (WKY, $\circ$) rats and quinapril-treated WKY rats (quin-WKY, $\bullet$). The responses were induced in the (a) absence and presence (b) of 0.1 mM N^G-nitro-L-arginine methyl ester (L-NAME). The insert (SHR $\blacksquare$, quin-SHR $\hatchedbox$, Wky $\square$, quin-WKY $\hatchedbox$) shows the increase in pD₂₅ value (i.e. sensitivity) induced by L-NAME, pD₂₅ is the negative logarithm of concentration of agonist inducing 25% response when compared to maximum. Symbols indicate means with s.e.means, $n=10-12$ in each group; * $P<0.05$, ANOVA for repeated measurements.

Table 2 Parameters of contractile responses to noradrenaline in isolated mesenteric arterial rings

	SHR	QSHR	WKY	QWKY
<i>Noradrenaline</i>				
pD ₂₅				
+ E	7.11 ± 0.17	6.38 ± 0.16*	6.84 ± 0.08	6.98 ± 0.16
+ E with L-NAME	7.44 ± 0.16	7.30 ± 0.21	7.43 ± 0.16	7.73 ± 0.12
-E	7.34 ± 0.25	7.68 ± 0.25	7.58 ± 0.22	7.45 ± 0.13
<i>Maximal force (g)</i>				
+ E	2.73 ± 0.26	1.40 ± 0.22*	2.27 ± 0.27†	2.01 ± 0.27
+ E with L-NAME	2.86 ± 0.29	1.74 ± 0.26*	2.45 ± 0.18†	2.24 ± 0.24
-E	2.21 ± 0.24	1.13 ± 0.27*	1.68 ± 0.43	1.82 ± 0.14*

Values are mean ± s.e.mean, $n=10-12$ in each group. SHR and QSHR, untreated and quinapril-treated spontaneously hypertensive rats, respectively; WKY and QWKY, untreated and quinapril-treated Wistar-Kyoto rats, respectively. +E and -E, endothelium-intact and -denuded mesenteric arterial rings, respectively. pD₂₅ is the negative logarithm of the concentration of agonist producing 25% of maximal contractile response. * $P<0.05$ compared with SHR group, † $P<0.05$ compared with QSHR group (Bonferroni test).

quinapril-treated SHR than in untreated SHR, which is in agreement with previous findings (Clozel *et al.*, 1990; Lee *et al.*, 1991; Major *et al.*, 1993). Thus, ACE inhibitor treatment reduced noradrenoceptor-mediated vasoconstriction. However, in endothelium-intact rings also sensitivity to NA was lower in quinapril-treated SHR than in untreated SHR, whereas in the presence of L-NAME no significant differences in the contractile sensitivity were found between the study groups. Thus, endothelium via NO release more effectively modulated arterial contractile sensitivity in quinapril-treated than in untreated SHR. On the basis of previous work, NA is known to release EDRF via the activation of α_2 -receptors on the endothelium (Cocks & Angus, 1983), and promoted NO production could also lead to more effective inactivation of endothelium-derived contractile factors (Auch-Schwelk *et al.*, 1992b).

In conclusion, long-term quinapril therapy improved relaxations to ACh and ADP in NA-precontracted mesenteric arterial rings of SHR in the absence and presence of NO synthesis inhibition, while the responses under conditions in which endothelium-dependent hyperpolarization was pre-

vented, were comparable between the study groups. Therefore, enhanced endothelium-mediated relaxations to agonists can be attributed to promoted endothelium-dependent hyperpolarization in quinapril-treated SHR. On the other hand, the potentiation of the response to ACh by exogenous bradykinin in quinapril-treated SHR, as well as the increased attenuating effect of the endothelium on NA-induced contractions in these animals, appeared to result from enhanced endothelium-derived NO release. Taken together, long-term ACE inhibition effectively restored the impaired endothelial function in SHR.

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