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Quinapril treatment and arterial smooth muscle responses in spontaneously hypertensive rats

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1 The effects of long-term angiotensin-converting enzyme inhibition with quinapril on arterial function were studied in spontaneously hypertensive rats, Wistar-Kyoto rats serving as normotensive controls.

2 Adult hypertensive animals were treated with quinapril (10 mg kg^{-1} day^{-1}) for 15 weeks, which reduced their blood pressure and the concentrations of atrial natriuretic peptide in plasma and ventricular tissue to a level comparable with that in normotensive rats.

3 Responses of mesenteric arterial rings *in vitro* were examined at the end of the study. Compared with normotensive and untreated hypertensive rats, responses to noradrenaline were attenuated in hypertensive animals on quinapril, both force of contraction and sensitivity being reduced. Quinapril also attenuated maximal contractions but not sensitivity to potassium chloride. Nifedipine less effectively inhibited vascular contractions in normotensive and quinapril-treated than in untreated hypertensive rats.

4 Arterial relaxation responses by endothelium-dependent (acetylcholine) and endothelium-independent (sodium nitrite, isoprenaline) mechanisms were similar in normotensive and quinapril-treated rats and more pronounced than in untreated hypertensive rats.

5 Cell membrane permeability to ions was evaluated by means of potassium-free solution-induced contractions of endothelium-denuded denervated arterial rings. These responses were comparable in normotensive and quinapril-treated rats and less marked than in untreated hypertensive rats.

6 Intracellular free calcium concentrations in platelets and lymphocytes, measured by the fluorescent indicator quin-2, were similar in normotensive and quinapril-treated rats and lower than in untreated hypertensive rats.

7 In conclusion, quinapril treatment improved relaxation responses and attenuated contractions in arterial smooth muscle of hypertensive rats. These changes may be explained by diminished cytosolic free calcium concentration, reduced cell membrane permeability, and alterations in dihydropyridine-sensitive calcium channels following long-term angiotensin-converting enzyme inhibition.

Keywords: Angiotensin-converting enzyme inhibition; atrial natriuretic peptide; arterial smooth muscle; blood pressure; quinapril; spontaneously hypertensive rats

Introduction

The principal mechanism of blood pressure reduction by angiotensin-converting enzyme (ACE) inhibitors is a fall in peripheral arterial resistance resulting from decreased angiotensin II formation (Frohlich, 1989). However, during chronic therapy, in particular, some discrepancy remains between the antihypertensive effect of ACE inhibitors and the suppression of systemic angiotensin II levels (Waeber *et al.*, 1989). Hence other mechanisms of blood pressure reduction may be operative during long-term ACE inhibition, namely decreased degradation of kinins, enhanced formation of vasodilator prostaglandins (Schrör, 1990), sympatho-inhibitory action, and inhibition of angiotensin II formation at tissue level (Frohlich, 1989).

During the past few years the vascular endothelium has been discovered to be an important regulator of both normal and pathological blood vessel tone (for a review, see Vane *et al.*, 1990 and Moncada *et al.*, 1991). ACE is largely located on the luminal surface of the arterial endothelium (Caldwell *et al.*, 1976). Moreover, 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). A short four day-long ACE inhibitor treatment has been shown to augment endothelium-dependent but not to affect endothelium-independent vascular relaxation in spontaneously hypertensive rats (SHR) (Clozel *et al.*, 1990). In addition, ACE inhibitors have been reported to increase the production of endothelium-derived relaxing factor(s) *in vitro* (Mombouli *et al.*, 1991). Wiemer *et al.* (1991) have recently shown that ramiprilat prevents the degradation of bradykinin liberated from the endothelial cells, which in turn stimulates the release of relaxing factors from the endothelium. This mechanism may partly explain the endothelium-mediated effects of ACE inhibitors.

Chronic ACE inhibition has been found to prevent effectively cardiac hypertrophy and to attenuate vascular responses to noradrenaline (NA) in SHR and Milan hypertensive rats (Lee *et al.*, 1991; Mulvany *et al.*, 1991), and to reduce pressor responses to infused NA also in man (Malini *et al.*, 1990). In addition it has been suggested that long-term ACE inhibition reduces arterial tone in SHR by reducing intracellular free calcium concentration ($[Ca^{2+}]_i$) in vascular smooth muscle (Sada *et al.*, 1990). Even brief 4-week ACE inhibitor therapy during the development of hypertension can induce a long-term blood pressure reduction in SHR, with associated permanent changes in vascular resistance properties. In contrast, when ACE inhibitor treatment is introduced in the established phase of hypertension, the vascular changes

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attained are transient and cardiovascular hypertrophy and enhanced resistance responses redevelop if therapy is withdrawn (Adams *et al.*, 1990; Harrap *et al.*, 1990).

In the present study, we elucidated the effects of long-term treatment with quinapril, an orally active non-sulphydryl compound (Kaplan *et al.*, 1984) inducing prolonged inhibition of ACE especially in vascular, heart and kidney tissues (Fabris *et al.*, 1990), on arterial responses in SHR. Quinapril therapy was started in the established phase of hypertension to examine if blood pressure normalization can reverse the abnormalities of arterial function found in this type of genetic hypertension. We investigated whether the altered responses to NA following ACE inhibition (Lee *et al.*, 1991; Mulvany *et al.*, 1991) are associated with changes in vascular calcium sensitivity or in sensitivity to other contractile agents. Moreover, the effects of long-term quinapril therapy on both endothelium-dependent and endothelium-independent vascular relaxation mechanisms were studied. Since the arterial smooth muscle contractile response induced by a K^+-free solution is known to differentiate clearly normotensive and hypertensive blood vessels (Arvola *et al.*, 1992), and is also an indirect method for the assessment of vascular permeability, this response was included in the study protocol. The efficacy of the quinapril treatment was evaluated by atrial natriuretic peptide (ANP) determinations from plasma and ventricular tissue, the concentrations of which are known to correlate positively with cardiac hypertrophy (Kinnunen *et al.*, 1990). Furthermore, platelets and lymphocytes were used as cell models to study the associated changes in $[Ca^{2+}]_i$.

Methods

Animals and experimental design

Male SHR of the Okamoto-Aoki strain and age-matched Wistar-Kyoto (WKY) rats were obtained from Møllegaard's Breeding Centre, Ejby, Denmark. The rats were housed 4–5 animals to a cage in a standard experimental animal laboratory (illuminated from 06 h 00 min to 18 h 00 min, room temperature + 22°C), and had free access to drinking fluid (tap water) and food pellets (Ewos, Södertälje, Sweden). At 8 weeks of age the SHR were divided into two groups of equal mean systolic blood pressures, which were measured from unanaesthetized rats by the tail cuff method at + 28°C (Model 129 Blood Pressure Meter; IITC Inc., Woodland Hills, Ca., U.S.A.). Beginning at the age of 15 weeks, SHR ($n = 10$) were given quinapril 0.12 mg ml^{-1} of drinking fluid (the average daily dose thus being 10 mg kg^{-1} body weight), while untreated SHR ($n = 12$) and normotensive WKY rats ($n = 10$) were kept on normal drinking fluid. Daily chow consumption was measured by weighing the pellets. Weekly indirect blood pressure measurements and the quinapril therapy continued for 15 more weeks until the animals were 30 weeks old. Thereafter, quinapril administration was withdrawn 1 day before the rats were decapitated, exsanguinated and blood samples drawn into polystyrene tubes containing heparin (100 units ml^{-1}) as anticoagulant for $[Ca^{2+}]_i$ determinations, and chilled tubes on ice containing EDTA (2.7 mM) as anticoagulant for plasma ANP assays. 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

Four successive 3-mm-long standard sections of the mesenteric artery were cut, beginning 0.5 cm distally from the mesenteric artery-aorta junction. The rings were placed between stainless steel hooks and mounted in an organ bath chamber 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 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 (FT03 transducer, Model 7 E Polygraph; Grass Instrument Co., Quincy, Ma., U.S.A.). The presence of intact endothelium in vascular preparations was confirmed by observing an almost complete relaxation response to 1 μM acetylcholine (ACh) in 1 μM NA-precontracted rings.

Vascular preparation 1: After a 30 min stabilization period, concentration-response curves for NA and 30 min later for potassium chloride (KCl), were cumulatively determined from the most proximal section of each mesenteric artery. In solutions containing high concentrations of potassium (20–125 mM), NaCl was replaced with KCl on an equimolar basis.

Vascular preparation 2: The next vascular ring was used to study the effect of organ bath calcium concentration on NA-induced contractions, i.e. the calcium-sensitivity of the rings during α-adrenoceptor agonist stimulation. A modification of the method described by Mulvany & Nyborg (1980) was applied. Briefly, after 30 min of stabilization the calcium stores of vascular rings were depleted by bathing them in a Ca^{2+}-free PSS for 40 min and by twice contracting them maximally with 10 μM NA during this period. Thereafter 10 μM NA-induced contractions were repeatedly elicited in increasing organ bath calcium concentrations (0.01–2.5 mM). After each contraction had developed fully, NA was rinsed from the baths with PSS containing the next concentration of calcium to be studied, the following contraction to 10 μM NA being induced in 2 min. When this procedure was completed, the same scheme of repeated 10 μM NA-induced contractions in increasing organ bath calcium concentrations (0.01–2.5 mM) was elicited in the presence of 0.5 nM nifedipine.

Vascular preparation 3: Vascular relaxation responses to ACh, isoprenaline and sodium nitrite ($NaNO_2$) were examined in the third section of each mesenteric artery. The rings were precontracted with 1 μM NA, and after the contraction had developed fully increasing concentrations of relaxing agent were added cumulatively to the organ bath. The next concentration of relaxing compound was added only after the previous level of relaxation was stable. The rings were allowed a 20-min recovery period in resting tension between the study of each relaxant.

Vascular preparation 4: Vascular endothelium was denuded from the most distal section of each mesenteric artery by gently rubbing with a jagged injection needle (Arvola *et al.*, 1992). The absence of endothelium was confirmed with a complete lack of relaxation to 1 μM ACh in 1 μM NA-precontracted vascular rings. Thereafter chemical sympathectomy was performed by the method described by Aprigliano & Hermsmeyer (1976): the rings were denervated *in vitro* by exposing them to a buffer-free solution containing 1.2 mM 6-hydroxydopamine, and by vigorous gassing with nitrogen for 15 min, followed by a 2-h recovery period in normal PSS. After this maximal 125 mM KCl-induced contractions (serving as reference responses) were elicited, and after another 30 min the rings were exposed to K^+-free buffer solution (pH 7.4), which was prepared by substituting KH_2PO_4 and KCl of normal PSS with NaH_2PO_4 and NaCl, respectively, on an equimolar basis. The omission of potassium induced gradual contractions in all vascular rings. After the contraction had reached a plateau, 1 mM potassium was re-added and the subsequent relaxation registered.

After registration of responses all preparations were dried

overnight at + 100°C and weighed. Vascular contractile responses were related to tissue dry weight. NA-induced contractions in different organ bath calcium concentrations were normalized by relating them to the maximal contractile response in 2.5 mM calcium in each arterial ring. The effect of nifedipine on NA-induced contractions in different organ bath calcium concentrations was calculated by relating the response in the presence of nifedipine to that without it. The relaxations were presented as percentage of pre-existing contractile force. In each ring EC_{50} was calculated from plots of the concentration of contractile agent versus the percentage of maximal response, or from plots of the concentration of relaxing compound versus the percentage of 1 μM NA-induced precontraction. The mean relaxation rate after maximal responses to 125 mM KCl was calculated from force of contraction and the time required to return to resting tension after rinsing the vascular rings with normal PSS. The contractions induced by K^+-free medium and the relaxation responses following the re-addition of potassium were normalized by relating them to the previously determined maximal KCl-induced (125 mM) contraction or tissue dry weight in each ring. After potassium repletion, the greatest reduction in smooth muscle contractile force during a 1 min period was considered the maximal relaxation rate.

Morphological studies

From six animals in each study group, vascular rings were prepared for light microscopy from the most proximal part of the remaining section of each mesenteric artery. The rings were fixed in 2% glutaraldehyde at + 4°C and postfixed in 2% osmiumtetroxide. After washing, they were stained with 1% uranyl acetate and dehydrated with acetone series. Thereafter the samples were embedded in Epon (LX-112 Resin, Ladd, Burlington, Vt., U.S.A.). Thin (2 μm) transverse sections were stained with 1% toluidine blue, and examined and photographed under light microscopy (Nikon Microphot-FXA, Japan). In each vascular ring the thickness of medial smooth muscle (determined as the mean medial thickness of four arterial wall quadrants) was measured from the photographs.

Atrial natriuretic peptide determinations

Left and right auricles were removed to be used for atrial ANP measurements, and the remaining atrial tissue was carefully cut separate from ventricular tissue. The ventricles were cut into superior (about 15–20% of total weight) and inferior parts, the latter being used for ventricular ANP determinations to avoid contamination of atrial tissue in these samples. All samples were blotted dry, weighed, and stored at − 70°C until assayed.

Blood samples were centrifuged (2,000 *g*, 10 min, at + 4°C), and ANP extracted from plasma as previously described (Ruskoaho *et al.*, 1989). ANP extraction from heart tissue was performed by homogenizing the atrial and ventricular samples with 3 ml of 0.3 M HCl as previously described (Ruskoaho & Leppäluoto, 1988). For the radioimmunoassay (RIA) the atrial and ventricular extracts were diluted 3×10^5 and 1.2×10^3 fold, respectively, with the RIA buffer. Then the tissue extracts and the plasma samples were incubated in duplicates of 100 μl with 100 μl of the specific rabbit ANP antiserum in the final dilution of $1{:}2.5 \times 10^4$. ANP was determined from plasma and tissue samples by RIA as earlier described by Ruskoaho *et al.* (1989). Tissue ANP is expressed as concentration per milligram tissue wet weight.

Measurement of intracellular free calcium in platelets and lymphocytes

The cytosolic free calcium concentration was measured as previously described (Pörsti *et al.*, 1992). Briefly, whole blood was diluted 1:2 with RPMI-1640 medium (Flow Laboratories, Irvine, Scotland, U.K.) and 4 ml of the sample was layered on 3 ml of Ficoll-Paque solution (Pharmacia, LKB Biotechnology Inc., Piscataway, N.J., U.S.A.). After centrifugation (25 min, 120 *g*, + 20°C) the plasma was removed and platelets and lymphocytes were harvested from plasma-Ficoll interface. The cells were resuspended in 4 ml of RPMI-1640 medium, and 0.4 ml of ACD solution (8% citric acid, 2.2% trisodium citrate, 2.45% dextrose, pH 6.5) was added. Platelets and lymphocytes were separated by centrifugation (10 min, 100 *g*, + 20°C), and lymphocytes were washed with RPMI-1640 and resuspended in HEPES medium (NaCl 145, $MgSO_4$ 5, glucose 10 and HEPES 10 mM; pH 7.4). The platelets were spun down from the supernatant (10 min, 500 *g*, + 20°C), washed twice with HEPES, and finally resuspended in this medium. The cell suspensions ($1-2 \times 10^9$ platelets ml^{-1}, $3-4 \times 10^6$ lymphocytes ml^{-1}) were loaded with 50 μM acetoxymethyl ester of quin-2 for 30 min at + 37°C, and washed twice with HEPES to remove extracellular quin-2. Finally the cells were resuspended in + 37°C Hanks' balanced salt solution ($2-2.5 \times 10^8$ platelets ml^{-1}; $1-2 \times 10^6$ lymphocytes ml^{-1}) containing 1.1 mM calcium, and fluorescence was recorded with a Shimadzu RF-5000 spectrofluorometer (Shimadzu Corp., Kyoto, Japan). The excitation and emission wavelengths were set at 339 nm and 492 nm, respectively. Calibration of the signal was performed basically according to the method described by Tsien *et al.* (1982). Maximal fluorescence was measured after 2 μM ionomycin and 5 mM $CaCl_2$ were added to the solution; minimal fluorescence after 25 mM Tris-EGTA (pH 8.6) and 0.1% Triton X100 were added.

Drugs

The following drugs were used: quinapril hydrochloride (Warner-Lambert Co., Ann Arbor, Mi., U.S.A.), acetylcholine chloride, calcium salt of ionomycin, 6-hydroxydopamine hydrobromide, isoprenaline hydrochloride, ammonium salt of heparin, synthetic rat ANP_{1-28} (Sigma Chemical Co., St. Louis, Mo., U.S.A.), acetoxymethyl ester of quin-2, Triton X100 (Aldrich Chemical Co., Milwaukee, Wis., U.S.A.), noradrenaline hydrogentartrate (Fluka Chemie AG, Buchs, Switzerland), nifedipine (Orion Pharmaceutical Co., Espoo, Finland) and sodium nitrite (E. Merck AG, Darmstadt, Germany). 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 ionomycin (in absolute ethanol), quin-2 (in dimethylsulphoxide), nifedipine (in 50% ethanol) and 6-hydroxydopamine (directly in buffer-free PSS). All solutions were freshly prepared before use and protected from light.

Statistics

Statistical analysis was carried out using one-way analysis of variance (ANOVA), supported by Bonferroni confidence intervals in the case of pairwise comparisons between the study groups. When the data consisted of repeated observations at successive time points analysis of variance for repeated measurements was applied. The results are expressed as mean ± s.e.mean. The data were analyzed with BMDP Statistical Software (Los Angeles, Ca., U.S.A.).

Results

Blood pressure, heart weight, plasma and tissue ANP concentrations, intracellular free calcium, and body weight

The systolic blood pressure of SHR was already higher at the beginning of the study than in WKY rats. During the 7-week

period before quinapril administration, blood pressures in the two SHR groups were elevated equally. Quinapril treatment beginning at the age of 15 weeks reduced blood pressure in SHR to normotensive level during the follow-up period, while in untreated SHR the rise in blood pressure continued (Figure 1 and Table 1). Cardiac hypertrophy was totally prevented in SHR by quinapril, relative heart weights of treated SHR not differing from those of WKY rats. Platelet and lymphocyte $[Ca^{2+}]_i$, measured by the fluorescent indicator quin-2, were comparable in WKY rats and quinapril-treated SHR, and significantly lower than in untreated SHR (Table 1). In addition, quinapril therapy reduced plasma and ventricular ANP, the concentrations in treated SHR being comparable to those in WKY rats and markedly lower than in untreated SHR (Figure 2). The total amounts of ANP in the ventricular tissue of untreated SHR, quinapril-treated SHR, and WKY rats were 157.4 ± 10.0***, 38.3 ± 5.4**,††† and 70.0 ± 5.6 ng, respectively (**$P<0.01$; ***$P<0.001$ compared with WKY rats; †††$P<0.001$ versus SHR). ANP content in the right atrium was similar in all groups, the concentration in the left atrium being higher in quinapril-treated than in untreated SHR (Figure 2).

SHR 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 (e.g. in appearance, activity, behaviour) were observed by our experienced experimental animals laboratory staff. In addition, average daily chow consumption was similar in the SHR groups: 11 ± 3 and 12 ± 3 g kg^{-1} body weight daily in treated and untreated SHR, respectively.

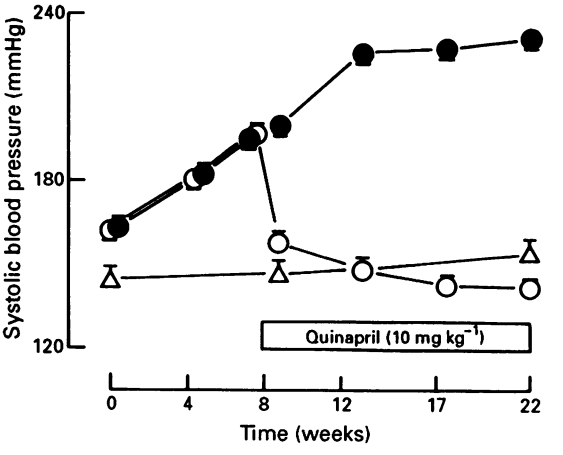

Figure 1 Systolic blood pressures in untreated spontaneously hypertensive rats (SHR, ●), SHR treated with quinapril (○) and untreated normotensive Wistar-Kyoto rats (WKY, △). The 15 week-long quinapril treatment (10 mg kg^{-1} body weight per day; indicated by the horizontal bar) of SHR was initiated at the age of 15 weeks and it effectively lowered blood pressure ($P<0.001$ versus untreated SHR, ANOVA for repeated measurements). Final systolic blood pressures did not significantly differ between quinapril-treated SHR and untreated WKY rats. Symbols indicate means with s.e.mean, $n = 10-12$ in each group.

Mesenteric arterial responses in vitro

When related to tissue dry weight, NA-induced maximal contractile force in isolated arterial rings was clearly lower in untreated SHR than WKY rats. However, 3-mm-long vascular rings from SHR, excised from a standard section of mesenteric artery, weighed more than those from WKY rats, and without relation to tissue weight maximal contractions were similar in these two strains. Untreated SHR had higher sensitivity to NA, i.e. lower EC_{50} values, than WKY rats (Figure 3 and Table 2). Contractile force generation to NA was reduced in SHR by quinapril therapy and was markedly lower than in WKY rats, whether related to tissue dry weight or not. In addition, sensitivity to NA was lowest in SHR on quinapril. Vascular rings of quinapril-treated SHR, too, weighed more than in WKY rats (Figure 3 and Table 2). The tissue weight-related contractile force induced by high concentrations of KCl was also lower in untreated SHR than WKY rats, and without the relation maximal contractions were again similar in these groups. SHR on quinapril showed clearly attenuated KCl-induced contractions when compared with WKY rats, but only differed from untreated SHR at high KCl concentrations (80–125 mM). Sensitivity to KCl was comparable in untreated SHR and WKY rats, but was slightly higher in quinapril-SHR than in the other groups (Figure 3 and Table 2).

The effect of organ bath calcium concentration on 10 μM NA-induced contractions, i.e. the calcium sensitivity of the vascular rings during α-adrenoceptor stimulation, was similar in all three study groups (Figure 4). However, the inhibitory effect of 0.5 nM nifedipine on NA-evoked contractions in different organ bath calcium concentrations was lower in

Table 1 Blood pressure, body weight and heart weight during the study, and intracellular free calcium concentrations at the close of the 22-week study

Variable	*Group* SHR	QSHR	WKY
Systolic blood pressure (mmHg)			
week 0	164 ± 2*	162 ± 3*	145 ± 4
week 7	196 ± 2***	197 ± 2***	149 ± 4
week 22	232 ± 3***	142 ± 3†††	155 ± 5
Body weight (g)			
week 0	151 ± 3	158 ± 5	159 ± 6
week 7	316 ± 4	316 ± 5	324 ± 6
week 22	412 ± 5	377 ± 5***†††	430 ± 7
Heart weight (g kg^{-1} body weight)	3.5 ± 0.1***	2.8 ± 0.1†††	2.9 ± 0.1
Intracellular free calcium concentration (nM)			
Platelets	105 ± 2***	91 ± 3†	83 ± 4
Lymphocytes	115 ± 3***	90 ± 4†††	94 ± 4

Values are mean ± s.e.mean; $n = 10-12$ in each group. SHR and QSHR, untreated and quinapril-treated spontaneously hypertensive rats, respectively; WKY, Wistar-Kyoto rats. The 15-week long quinapril treatment commenced at study week 7 when the animals were 15 weeks old.

*$P<0.05$, ***$P<0.001$ compared with WKY rats, †$P<0.05$, †††$P<0.001$ QSHR versus SHR (Bonferroni test).

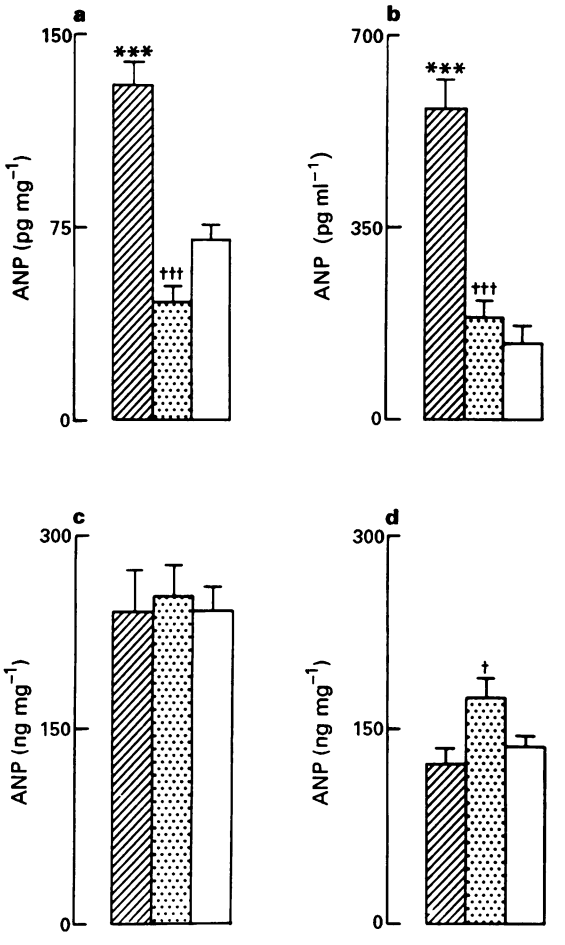

Figure 2 Atrial natriuretic peptide (ANP) concentrations in ventricular tissue (a), plasma (b), and in right (c) and left atrial tissues (d) of untreated spontaneously hypertensive rats (SHR, hatched columns), SHR treated with quinapril (stippled columns) and untreated Wistar-Kyoto rats (WKY, open columns). Columns indicate means with s.e.mean, $n = 6-10$ in each group. Statistical analysis was by one-way ANOVA followed by the Bonferroni test for individual pairs: ***$P<0.001$ compared with WKY rats; †$P<0.05$; †††$P<0.001$ quinapril-treated SHR versus untreated SHR.

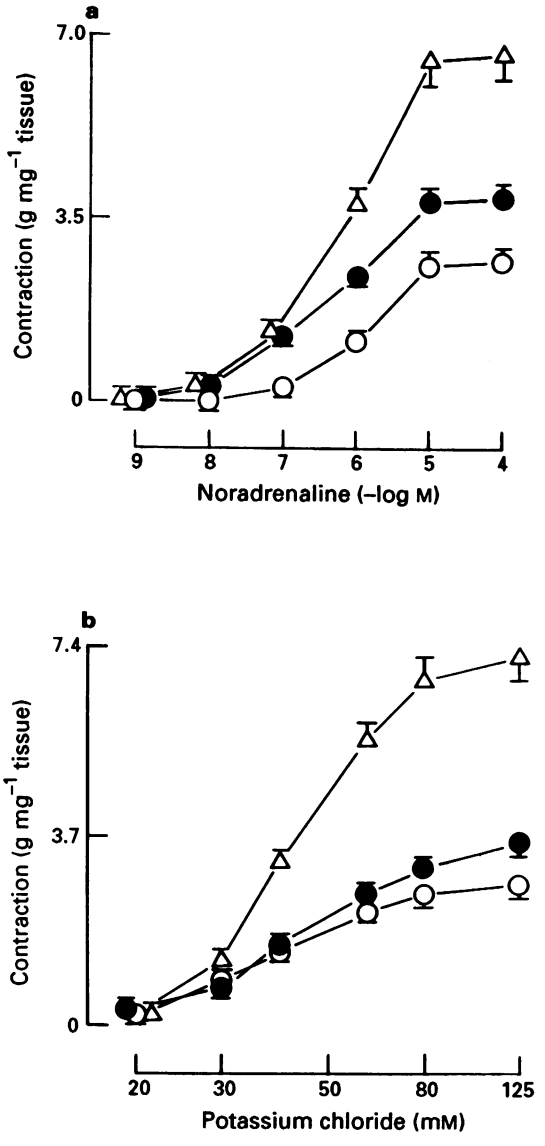

Figure 3 Contractile responses (related to tissue dry weight) of isolated endothelium-intact mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR, ●), SHR treated with quinapril (○) and untreated Wistar-Kyoto rats (WKY, △). (a) Cumulative concentration-response curves to noradrenaline. Contractile force generation was lower in SHR than WKY rats ($P<0.001$). Quinapril-treated SHR showed reduced contractions when compared with untreated SHR ($P<0.01$) and WKY rats ($P<0.001$). (b) Cumulative concentration-response curves to high concentrations of potassium chloride. Contractile force generation was again lower in SHR than WKY rats ($P<0.001$), and quinapril-treated SHR showed reduced contractions when compared with WKY rats ($P<0.001$), but differed from untreated SHR only at the highest concentrations of potassium chloride (80–125 mM, $P<0.01$). Symbols indicate means with s.e.mean, $n = 8-10$ in each group. Statistical analysis was by ANOVA for repeated measurements.

WKY rats and quinapril-treated SHR than in untreated SHR (Figure 4).

The relaxation responses of endothelium-intact mesenteric arterial preparations induced by ACh, $NaNO_2$, and isoprenaline were greatly impaired in untreated SHR when compared with WKY rats. All these relaxations were markedly improved in SHR by quinapril treatment, the responses not differing from those of WKY rats. High concentrations of ACh (10 μM) induced slight recontractions in untreated SHR, such phenomena being absent in WKY rats and quinapril-treated SHR (Figure 5 and Table 3). In addition, the rate of relaxation after washout of maximal (125 mM) KCl-induced contractions was similar in WKY rats and quinapril-SHR and markedly faster than in untreated SHR (Table 3).

In endothelium-denuded mesenteric arterial rings subjected to denervation *in vitro* with 6-hydroxydopamine, the time to onset of K^+-free solution-induced contraction was longer and the plateau phase of the response was attained later in WKY rats and quinapril-SHR than in untreated SHR. When related either to the 125 mM KCl-induced reference contractions or to tissue weight, maximal K^+-free responses were lower in WKY rats and quinapril-SHR than in untreated SHR (Figure 6 and Table 4). The potassium relaxation response of endothelium-denuded denervated arterial rings, induced by re-addition of 1 mM potassium upon maximal K^+-free contractions, was markedly impaired in untreated SHR when compared with WKY rats. Quinapril therapy clearly improved this response, the rate of potassium relaxation not differing between quinapril-SHR and WKY rats (Figure 6 and Table 4).

Morphological studies

Medial smooth muscle thickness, measured from mesenteric rings photographed under light microscopy, was moderately reduced in quinapril-treated SHR when compared with untreated SHR, but still remained clearly more pronounced

Table 2 Parameters of contractile responses and average medial thickness in isolated endothelium-intact mesenteric arterial rings

Variable	*Group* *SHR*	*QSHR*	*WKY*
EC_{50}			
Noradrenaline (nM)	419 ± 39*	1219 ± 254*††	731 ± 88
Potassium chloride (mM)	44 ± 1	37 ± 2*††	42 ± 1
Maximal force (g mg^{-1} tissue)			
Noradrenaline	3.9 ± 0.3***	2.7 ± 0.3***†	6.7 ± 0.5
Potassium chloride	3.7 ± 0.2***	2.8 ± 0.2***†	7.2 ± 0.5
Maximal force (g)			
Noradrenaline	2.7 ± 0.2	1.6 ± 0.1**††	2.5 ± 0.2
Potassium chloride	2.4 ± 0.1	1.7 ± 0.1***††	2.7 ± 0.1
Vascular ring dry weight (mg)	0.69 ± 0.04***	0.63 ± 0.06*	0.38 ± 0.03
Average medial thickness (μm)	117 ± 3***	103 ± 3***†	77 ± 4

Values are mean ± s.e.mean, $n = 8-10$ in each group, $n = 6$ for morphological studies. SHR and QSHR, untreated and quinapril-treated spontaneously hypertensive rats, respectively; WKY, Wistar-Kyoto rats. EC_{50} is the concentration of agonist producing 50% of maximal contractile response.
*$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with WKY rats, †$P<0.05$, ††$P<0.01$ QSHR versus SHR (Bonferroni test).

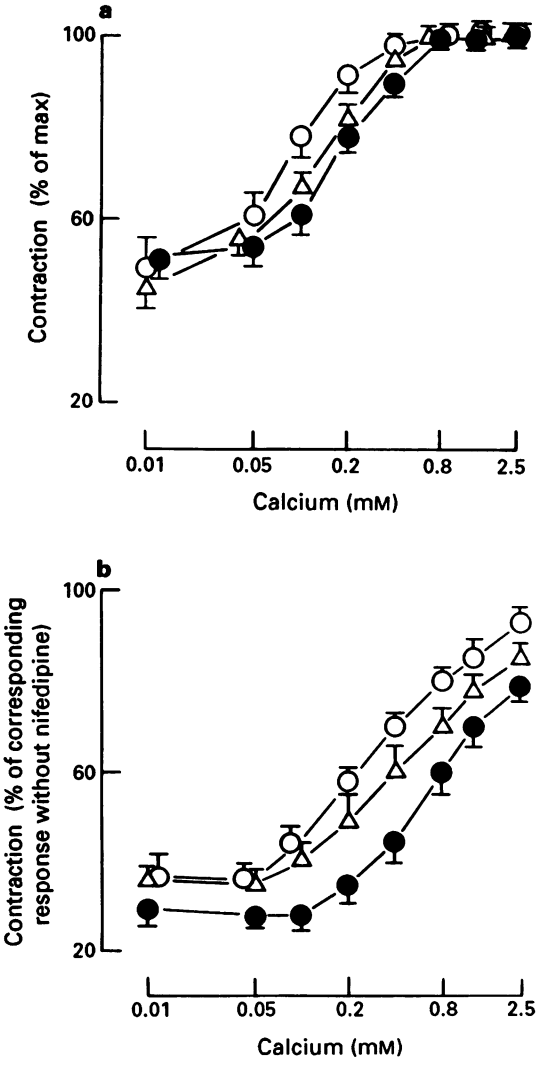

Figure 4 Contractile responses of isolated endothelium-intact mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR, ●), SHR treated with quinapril (○) and untreated Wistar-Kyoto rats (WKY, △). (a) Effect of organ bath calcium concentration on 10 μM noradrenaline-induced contractions. No significant differences were found between the groups. (b) Effect of 0.5 nM nifedipine on 10 μM noradrenaline-induced contractions in different organ bath calcium concentrations. Nifedipine inhibited contractions of WKY rats ($P<0.05$) and quinapril-treated SHR ($P<0.01$) less effectively than those of untreated SHR. Symbols indicate means with s.e.mean, $n = 8-10$ in each group. Statistical analysis was by ANOVA for repeated measurements.

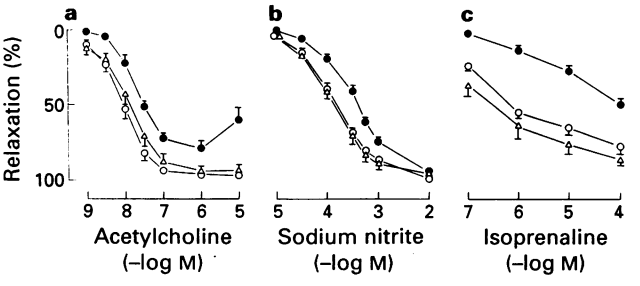

Figure 5 Cumulative relaxation responses of isolated endothelium-intact mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR, ●), SHR treated with quinapril (○) and untreated Wistar-Kyoto rats (WKY, △). Relaxations were induced by acetylcholine (a), sodium nitrite (b) and isoprenaline (c) after full precontraction with 1 μM noradrenaline. Quinapril-treated SHR and untreated WKY rats showed more pronounced relaxations than untreated SHR ($P<0.05$ for acetylcholine, $P<0.001$ for sodium nitrite and isoprenaline). Symbols indicate means with s.e.mean, $n = 8-10$ in each group. Statistical analysis was by ANOVA for repeated measurements.

than in WKY rats (Figure 7 and Table 2). The results of medial thickness in the study groups paralleled well with vascular ring dry weights (Table 2).

Discussion

In the present study, blood pressure was totally normalized and cardiac hypertrophy abolished in SHR by quinapril therapy, the results being consistent with previous investigations using other ACE inhibitors (Lee *et al.*, 1991; Frohlich & Horinaka, 1991). The renin-angiotensin-aldosterone system thus seems to be one of the most important factors supporting elevated blood pressure in SHR. Recent investigations have shown that the pathogenesis of hypertension in SHR may be related to the renin gene (Kurtz *et al.*, 1990; Morris, 1991). Given the suggested central role of the renin gene, it is

Table 3 Parameters of relaxation of isolated endothelium-intact mesenteric arterial rings

Variable	*Group* SHR	QSHR	WKY
EC_{50}			
Acetylcholine (nM)	27 ± 4*	11 ± 3††	12 ± 2
Sodium nitrite (μM)	459 ± 55***	165 ± 26†††	174 ± 44
Isoprenaline (μM)	94 ± 22***	1.2 ± 0.6†††	0.9 ± 0.3
Maximal relaxation (% of 1 μM noradrenaline -induced precontraction)			
Acetylcholine	79 ± 5*	97 ± 2††	94 ± 3
Sodium nitrite	95 ± 2	99 ± 1	96 ± 2
Isoprenaline	53 ± 4**	77 ± 5†	87 ± 4
Mean rate of relaxation after washout of 125 mM KCl (g mg^{-1} tissue min^{-1})	0.27 ± 0.02***	0.90 ± 0.11†††	1.08 ± 0.11

Values are mean ± s.e.mean, $n = 8-10$ in each group. SHR and QSHR, untreated and quinapril-treated spontaneously hypertensive rats, respectively; WKY, Wistar-Kyoto rats. EC_{50} is the concentration producing 50% relaxation of 1 μM noradrenaline-induced precontraction.
*$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with WKY rats, †$P<0.05$, ††$P<0.01$, †††$P<0.001$ QSHR versus SHR (Bonferroni test).

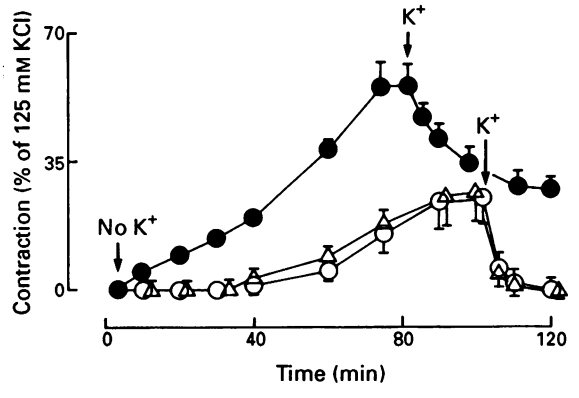

Figure 6 Contractile responses induced by K^+-free buffer solution (100% contraction represents the force attained by 125 mM KCl) and the subsequent relaxation responses to re-addition of 1.0 mM K^+ in isolated endothelium-denuded and sympathectomized mesenteric arterials rings from untreated spontaneously hypertensive rats (SHR, ●), SHR treated with quinapril (○) and untreated Wistar-Kyoto rats (WKY, △). The responses of WKY rats and quinapril-treated SHR did not differ, the rate of contractile force development being slower ($P<0.01$) and the rate of relaxation being faster ($P<0.05$) than in untreated SHR. Symbols indicate means with s.e.mean, $n = 6$ in each group. Statistical analysis was by one-way ANOVA followed by the Bonferroni test for individual pairs.

not surprising that inhibition of angiotensin II formation, the major effector of the renin-angiotensin system, was highly effective in lowering blood pressure and remodelling arterial function in the present study. Achieved normotension and reduced cardiac afterload also clearly reduced ventricular tissue ANP concentrations, which probably reflects diminished synthesis and storage of ANP, since hypertension-induced ventricular hypertrophy in SHR is known to be associated with increased synthesis, storage and release of ANP (Arai *et al.*, 1988; Ruskoaho *et al.*, 1989). The finding that plasma ANP was similar in WKY rats and quinapril-SHR and markedly lower than in untreated SHR supports this conclusion. Elevated ANP content in the left atrium of SHR on quinapril, in turn, probably results from reduced ANP release, since the rate of ANP synthesis in atrial tissue of SHR is known to be constant (Kinnunen *et al.*, 1991). Thus, the results on plasma and tissue ANP concentrations confirm the normalization of blood pressure in quinapril-treated SHR.

Weight gain was somewhat attenuated in SHR on oral quinapril, although chow consumption was comparable to that in untreated SHR. Previously, treatment with various other ACE inhibitors has been reported to result in reduced body weight in hypertensive rats (Adams *et al.*, 1990; Harrap *et al.*, 1990, Mulvany *et al.*, 1991). The explanation of this phenomenon remains unclear. However, in addition to other

Table 4 Parameters of K^+-free buffer solution-induced contractions and subsequent relaxations induced by 1.0 mM potassium in isolated endothelium-denuded and sympathectomized mesenteric arterial rings

Variable	*Group* SHR	QSHR	WKY
Contraction to 125 mM KCl (g mg^{-1} tissue)	4.6 ± 0.4	4.2 ± 0.4	4.9 ± 0.6
Potassium-free contraction			
Time to onset (min)	4.2 ± 1.5**	51.0 ± 5.7††	46.4 ± 9.9
Time to maximum (min)	73.3 ± 3.7**	102.7 ± 6.5††	96.2 ± 6.3
Maximal force			
(g mg^{-1} tissue)	2.6 ± 0.3*	0.9 ± 0.2†	1.1 ± 0.4
(% of 125 mM KCl)	56.5 ± 7.2*	23.7 ± 7.3†	22.4 ± 7.6
Potassium relaxation rate			
(mg mg^{-1} tissue min^{-1})	87 ± 20*	354 ± 82†	504 ± 44
(% of 125 mM KCl min^{-1})	2.0 ± 0.5*	8.3 ± 2.0†	10.3 ± 0.9

Values are mean ± s.e.mean, $n = 6$ in each group. SHR and QSHR, untreated and quinapril-treated spontaneously hypertensive rats, respectively; WKY, Wistar-Kyoto rats.
*$P<0.05$, **$P<0.01$ compared with WKY rats, †$P<0.05$, ††$P<0.01$, QSHR versus SHR (Bonferroni test).

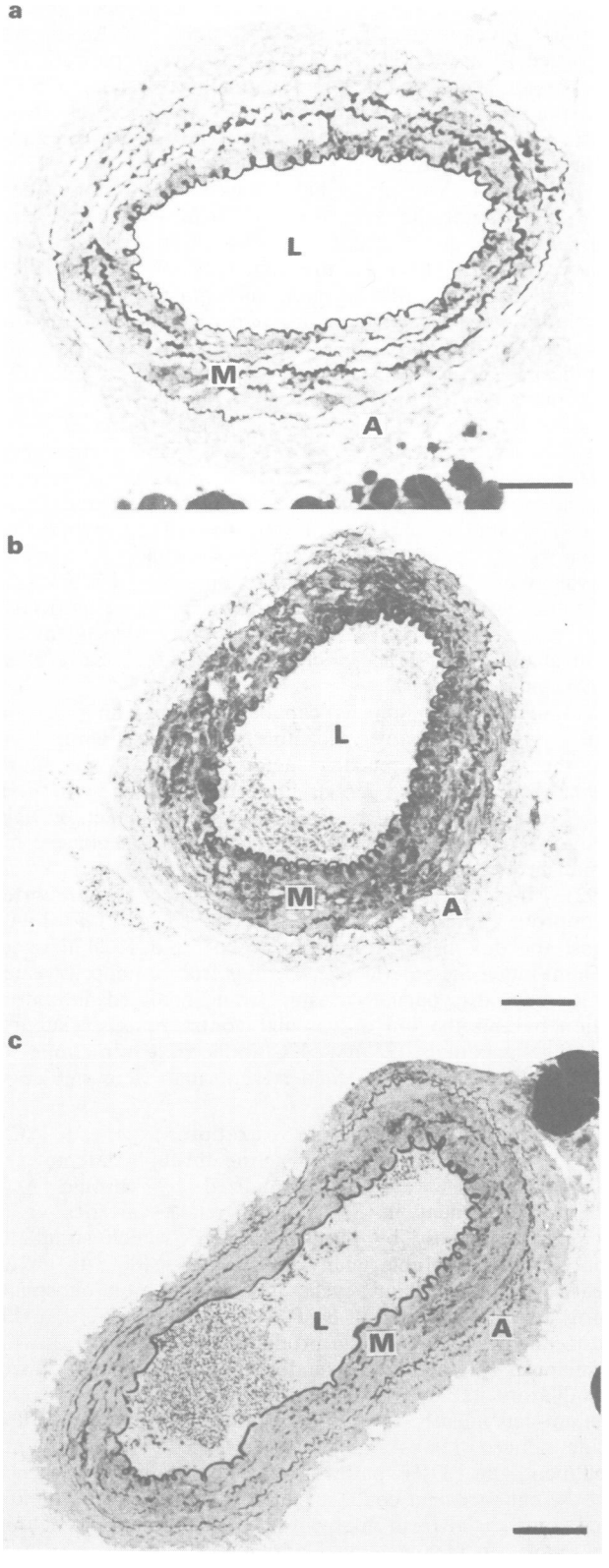

Figure 7 Light microscopy of representative mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR, a), SHR treated with quinapril (b) and untreated Wistar-Kyoto rats (c). A = adventitia, M = media, L = lumen; calibration bar = 100 μm.

functions, angiotensin II acts as a growth factor in the cardiovasular system (Dzau *et al.*, 1991). Moreover, enhanced responses to angiotensin II and other growth factors have been reported in both vascular smooth muscle cells and skin fibroblasts of SHR (Paquet *et al.*, 1989; Guicheney *et al.*, 1991). Thus, reduction of angiotensin II formation by ACE inhibitors may affect growth of vascular and nonvascular cells in experimental animals, especially in SHR. Attenuated weight gain may also result from unspecific effects on other factors affecting growth, or from reduced plasma aldosterone concentration and the subsequent natriuresis and diuresis following long-term ACE inhibition (Säynävälammi *et al.*, 1986).

The concentration of intracellular free calcium is a major factor determining arterial tone (Stull *et al.*, 1991). Vascular smooth muscle cells in SHR are characterized by abnormal calcium handling already at the prehypertensive stage: although basal $[Ca^{2+}]_i$ in 4-week-old animals is similar to that in WKY rats, stimulation with caffeine or angiotensin II results in higher values in SHR. In 8-week-old SHR with manifest hypertension basal $[Ca^{2+}]_i$, too, is higher than in WKY rats (Sugiyama *et al.*, 1990). Elevated $[Ca^{2+}]_i$ has also been found in platelets (Pörsti *et al.*, 1992) and lymphocytes of SHR (Furspan *et al.*, 1989). The possibility that elevated $[Ca^{2+}]_i$ in these circulating blood cells might only be a secondary phenomenon to high blood pressure and shear stress has raised concern (Young *et al.*, 1988). However, Oshima *et al.* (1991) reported that both basal and thrombin-stimulated $[Ca^{2+}]_i$ in platelets and concanavalin A-stimulated $[Ca^{2+}]_i$ in thymic lymphocytes of 4-week-old SHR are higher than in WKY rats. Thus, abnormalities in calcium metabolism appear to be prior events rather then subsequent to the development of hypertension in SHR, and elevated $[Ca^{2+}]_i$ can be found in circulating and noncirculating cells that are shielded from shear stress. In the present study, $[Ca^{2+}]_i$ was clearly higher in platelets and lymphocytes of SHR when compared with WKY rats, and was significantly reduced by quinapril therapy in both of these cell types in SHR. Sada *et al.* (1990) have recently reported that ACE inhibitor treatment both normalizes $[Ca^{2+}]_i$ and decreases Ca^{2+}-dependent tone in vascular smooth muscle of SHR. Thus, the reduction in blood pressure following long-term ACE inhibitors is associated with improved cellular calcium regulation in SHR.

In the present study, quinapril treatment of SHR was started when the animals were 15 weeks old and their systolic blood pressures had risen to nearly 200 mmHg, to examine whether long-term ACE inhibition at this advanced stage can reverse the abnormalities of arterial function associated with genetic hypertension. Morphological studies of the vasculature showed that medial smooth muscle hypertrophy was slightly reduced in treated SHR, but still remained clearly more pronounced than in WKY rats, in spite of the marked blood pressure reduction by quinapril. Thus, the hypertension-induced thickening of the mesenteric arterial wall could be only moderately reversed by the present ACE inhibitor treatment, corresponding to previous reports in which structural vascular changes in SHR could be effectively prevented only by very early introduction of antihypertensive therapy (Lee *et al.*, 1991).

The results presented here were obtained with selected segments of the mesenteric artery *in vitro* to study the effects of long-term ACE inhibition on arterial function. The fact that blood vessels from different parts of the vasculature may differ substantially must be kept in mind when relating the results to the whole vascular system. When smooth muscle contractions were induced by depolarizing the cell membrane with high concentrations of KCl, SHR showed comparable sensitivity but attenuated tissue weight-related force generation when compared with WKY rats. This type of 'forced' contraction depends mainly upon calcium influx through the voltage-dependent channels, a minor portion of the response resulting from the release of stored calcium (Johns *et al.*, 1987; Karaki & Weiss, 1988). We have previously reported that maximal tissue weight-related contractions induced by both high concentrations of KCl and NA are similar in SHR and WKY rats (Pörsti *et al.*, 1992). Those rats, however, were 10 weeks younger than the present 30-week-old animals, and an impairment of vascular contractions at the later and

more advanced stage of hypertension appears to be a likely explanation. Nonetheless, normalization of blood pressure in SHR by quinapril did not augment the KCl-induced contractile force generation of vascular rings to the level of WKY rats, but even further attenuated the response at higher KCl concentrations. Thus, the reduced arterial contractile response of 30-week-old SHR may be attributed rather to properties of the hypertrophied vascular wall than high blood pressure *per se*. Another possible explanation for these deviations in the results between different studies may be inborn differences between SHR and WKY rats, since recent investigations have shown clear genetic dissimilarities between these two strains (Johnson *et al.*, 1992; St. Lezin *et al.*, 1992).

In addition to cell membrane depolarization and the subsequent calcium entry, liberation of endogenous NA from adrenergic nerve-endings and α-adrenoceptor stimulation participate in the contractile response evoked by a high concentration of KCL (Xiao & Rand, 1991). The relative proportion of endogenous NA in KCl-induced contractions seems more pronounced in mesenteric rings from normotensive rats, since after denervation *in vitro* with 6-hydroxydopamine, maximal responses were no longer greater in WKY rats than in SHR. Moreover, ACE inhibition is known to eliminate the angiotensin II-induced facilitation of NA release in SHR (Adams *et al.*, 1990). Thus, the lower KCl-induced contractions in quinapril-treated SHR when compared with untreated SHR and WKY rats may also reflect diminished transmitter release from vascular adrenergic nerve endings.

Smooth muscle contractions were also induced by activating α-adrenoceptors with NA, which causes a rapid calcium release from the sarcoplasmic reticulum and prevents calcium re-uptake, the sustained contraction resulting from calcium influx through the receptor-linked channel (Johns *et al.*, 1987; Karaki & Weiss, 1988). Sensitivity to NA was highest in untreated SHR, corresponding to the elevated $[Ca^{2+}]_i$ in these animals. When compared with untreated SHR and WKY rats, both contractile force generation and sensitivity to NA were clearly reduced in quinapril-treated SHR. Freslon & Giudicelli (1983) and Lee *et al.* (1991) have reported attenuated responses to NA in captopril-treated SHR, the suggested mechanism being reduced vascular wall hypertrophy with lower smooth muscle mass following chronic ACE inhibition. Attenuation of pressor responses to infused NA has also been reported in lisinopril-treated essentially hypertensive patients (Malini *et al.*, 1990). The present results, however, do not support the view that ACE inhibitor therapy attenuates arterial contractions by diminishing smooth muscle mass, since medial thickness of the mesenteric artery was only slightly reduced. Moreover, reduced smooth muscle mass is not equivalent to smaller contractions, since normotensive WKY rats with clearly lower medial thickness (and vascular ring weight) showed more pronounced responses than SHR. Interestingly, vascular sensitivity to depolarization with KCl was slightly higher in SHR on quinapril than in untreated SHR and WKY rats. Thus the lower sensitivity to NA in quinapril-treated SHR suggests an effect of chronic ACE inhibition on postsynaptic α-adrenoceptor-mediated responses, the mechanism of which remains to be studied, but may be attributed to alterations in dihydropyridine-sensitive calcium channels (see below). In addition, differences in neuronal catecholamine uptake after ACE inhibition may have affected the results on mesenteric arterial NA sensitivity, since the present responses were not performed in the presence of a neuronal uptake inhibitor.

The fact that lower blood pressure alone does not explain reduced sensitivity to vasoconstrictors is supported by the study of Bund *et al.* (1991), who found increased sensitivity to NA in the parts of the arterial tree in SHR which were protected from hypertension by partial ligature. Recently Dohi *et al.* (1992) reported that perfusion with 0.1 μM angiotensin II stimulated the production of the potent vasoconstrictor peptide endothelin in SHR mesenteric arteries but not in those from WKY rats. Endothelin production, in turn, augmented pressor responses to NA, and was suggested to have an important role in the amplification of the pressor effects of the renin-angiotensin system in SHR. The possibility that quinapril-induced reduction of angiotensin II formation attenuates sensitivity to NA via reduced endothelin production remains to be studied.

The calcium sensitivity of NA-induced smooth muscle contractions was not altered by quinapril treatment in SHR and, moreover, no significant differences were found between SHR and WKY rats. However, the inhibitory effect of the dihydropyridine Ca^{2+}-entry blocker, nifedipine, on these contractions was lower in normotensive WKY rats and quinapril-treated SHR than in untreated SHR, suggesting significant differences in voltage-dependent calcium entry. We have previously reported that the arteries of SHR are more sensitive to effects of dihydropyridines than those of WKY rats (Arvola *et al.*, 1992; Pörsti, 1992). Rusch & Hermsmeyer (1988) have shown that the relative proportions of voltage-dependent calcium currents are different in SHR and WKY rats. The long-lasting current, sensitive to dihyropyridines (Bean *et al.*, 1986), is greater in SHR, while the transient dihydropyridine-resistant current predominates in WKY rats. Therefore, ACE inhibitor treatment seems to be associated with normalization of the function of voltage-dependent calcium channels in SHR, as suggested earlier by Sada *et al.* (1990).

Vascular endothelium is capable of controlling arterial tone by releasing many vasoactive substances, among them endothelium-derived relaxing factor (EDRF) (Vane *et al.*, 1990; Moncada *et al.*, 1991). Endothelial function in SHR has been reported to be impaired with smaller EDRF-mediated attenuation of vascular contractile reponses and diminished endothelium-dependent relaxation (Arvola *et al.*, 1992). Moreover, ACE inhibitor therapy has been reported to improve endothelial function in SHR (Clozel *et al.*, 1990). Thus, the diminished responses to NA and KCl in endothelium-intact mesenteric arterial rings from quinapril-treated SHR may also partially result from increased inhibitory action by endothelium or vascular contractions. In support of this, maximal 125 mM KCl-induced contractions of endothelium-denuded and denervated rings were similar in both SHR groups.

In the present study, vascular relaxation responses to ACh, $NaNO_2$ and isoprenaline, each acting through different cellular pathways, were all normalized by chronic ACE inhibitor treatment in SHR. ACh relaxes arteries endothelium-dependently by releasing EDRF, which stimulates smooth muscle soluble guanylate cyclase, leading to an increase in intracellular cyclic guanosine monophosphate (Moncada *et al.*, 1991). EDRF is probably nitric oxide (Moncada *et al.*, 1991), the production of which is also the mechanism of action of endothelium-independent nitrovasodilators like $NaNO_2$ (Feelish, 1991). Since both endothelium-dependently and -independently produced nitric oxide-induced relaxations were enhanced after quinapril treatment, the EDRF pathway may not be improved *per se* but the enhancement could result from changes in a common final route distal from guanylate cyclase. In support of this, the relaxation mediated by β-adrenoceptor stimulation with isoprenaline, leading to increased intracellular cyclic adenosine monophosphate, was also markedly improved by quinapril. In addition, the relaxation rate after washout of maximal (125 mM) KCl-induced contraction was comparable in WKY rats and quinapril-treated SHR and clearly faster than in untreated SHR. Since the concentration of intracellular free calcium, a major factor determining arterial tone (Stull *et al.*, 1991), was significantly reduced in SHR platelets and lymphocytes by quinapril, improved cellular calcium regulation remains a prime candidate to explain the augmented vascular relaxation responses. The discrepancy between the present relaxation results and the report of Clozel *et al.* (1990) who found that 4-days of cilazapril

therapy augmented relaxation responses to ACh but not to sodium nitroprusside in SHR, could result from the fact that endothelial function is first to recover following the reduction of blood pressure while prolonged therapy is required to induce alterations in vascular smooth muscle function.

Vascular contractions induced by Na^+, K^+-ATPase inhibition with a K^+-free medium in endothelium-denuded denervated arterial rings result from sodium leak to the cell, which causes depolarization and increases calcium influx through voltage-dependent channels (Mulvany, 1985). We have previously shown that the K^+-free contractions in these conditions are more pronounced in SHR when compared with WKY rats because of increased cell membrane permeability to sodium and enhanced voltage-dependent calcium entry (Arvola *et al.*, 1992). In the present study, the K^+-free contractions of SHR after chronic ACE inhibition appeared identical to those in WKY rats. Thus, long-term quinapril administration seems to be associated with normalization of arterial cell membrane permeability in SHR. Furthermore, vascular Na^+, K^+-ATPase activity was evaluated indirectly by re-addition of potassium upon full K^+-free medium-induced contractions. The return of potassium activates Na^+, K^+-ATPase, leading to cell membrane repolarization and thereby smooth muscle relaxation (Bonaccorsi *et al.*, 1977). In addition, the rate of potassium relaxation also reflects general relaxation mechanisms (e.g. contractile protein dephosphorylation, calcium sequestration and extrusion) (Johns *et al.*, 1987), but our previous results suggest that potassium relaxation rate is indicative of Na^+, K^+-ATPase activity in rat mesenteric arterial smooth muscle (Arvola *et al.*, 1992). The present results showed a normalized potassium relaxation rate in SHR after quinapril therapy, and thus suggest improved vascular Na^+K^+-ATPase activity following long-term ACE inhibition.

In conclusion, long-term ACE inhibitor therapy with quinapril effectively lowered blood pressure and abolished cardiac hypertrophy in SHR. The treatment was associated with normalization of (1) ANP concentrations in plasma and ventricular tissue, (2) arterial relaxation responses by endothelium-dependent and -independent mechanisms, (3) function of arterial dihydropyridine-sensitive calcium channels, (4) cell membrane permeability of vascular smooth muscle, and (5) concentration of intracellular free calcium in circulating platelets and lymphocytes. However, arterial contractile responses were not normalized but were attenuated in quinapril-treated SHR when compared with untreated SHR and WKY rats. The attenuated vascular contractions may be attributed to the prevailing vascular wall hypertrophy in spite of the considerable blood pressure reduction, while at the same time cellular calcium regulation and the functions of vascular endothelium, cell membrane and voltage-dependent calcium channels were normalized by quinapril in SHR. Thus, enhanced arterial relaxation and attenuated contractile responses may take part in the long-term antihypertensive effect of quinapril in this type of genetic hypertension.

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