Enhancement of arterial relaxation by long-term atenolol treatment in spontaneously hypertensive rats

¹Mika Kähönen, Heikki Mäkynen, Pertti Arvola & Ilkka Pörsti

Department of Biomedical Sciences, University of Tampere, P.O. Box 607, FIN-33101 Tampere, Finland

1 The effects of long-term atenolol (25 mg kg⁻¹ day⁻¹) therapy on arterial function were studied in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. The 14-week treatment attenuated the increase in blood pressure by approximately 30 mmHg in SHR, but did not affect blood pressure in WKY rats.

2 Responses of mesenteric arterial rings *in vitro* were examined at the end of the study. The relaxation to acetylcholine was similar in WKY rats and atenolol-treated SHR and more pronounced than in untreated SHR, whereas the relaxation to the nitric oxide donor 3-morpholinosydnonimine (SIN-1) was comparable in all study groups. Moreover, after maximal relaxations to acetylcholine, marked recontractions developed in untreated SHR but not in the other groups. Vasorelaxation to isoprenaline was also attenuated in SHR and was moderately improved by the atenolol therapy.

3 Arterial relaxation induced by return of potassium to the organ bath upon precontractions elicited by potassium-free solution were used to evaluate vascular smooth muscle Na⁺,K⁺-ATPase. The rate of potassium relaxation was fastest in WKY rats and was also faster in atenolol-treated than in untreated SHR.

4 The ability of vascular smooth muscle to sequester calcium was evaluated by eliciting responses to caffeine or noradrenaline after loading periods in different organ bath calcium concentrations. The subsequent contractions were lower in untreated SHR than in WKY rats, and augmented in SHR by the atenolol treatment.

5 Smooth muscle contractions to noradrenaline were comparable in SHR and WKY rats, while atenolol treatment slightly increased the maximal response to this agonist in SHR. Responses to potassium chloride were not affected by atenolol and contractions following cumulative re-addition of calcium to the organ bath after precontraction with potassium chloride and noradrenaline in calcium-free solution were comparable in all study groups.

6 In conclusion, the moderate antihypertensive effect of atenolol in SHR was accompanied by enhancement of β -adrenoceptor-mediated and normalization of endothelium-dependent arterial relaxation. Furthermore, ability to sequester calcium into cellular stores, and function of Na⁺,K⁺-ATPase were augmented in vascular smooth muscle. Therefore, the present results suggest that the long-term blood pressure-lowering action of atenolol in this type of genetic hypertension is accompanied by improved arterial relaxation and normalization of endothelial function.

Keywords: Arterial smooth muscle; atenolol; blood pressure; endothelium; spontaneously hypertensive rat

Introduction

The mechanisms beyond the antihypertensive action of β -adrenoceptor blockade have not been fully clarified, but reductions in adrenergic activity, cardiac output and renin release have been proposed to underlie the lowering of blood pressure (see Man in't Veld *et al.*, 1986; Meiracker *et al.*, 1989). Since different β -adrenoceptor blockers with quite dissimilar effects on the above variables have comparable effects on blood pressure, Man in't Veld *et al.* (1986) have suggested that the reductions observed in these factors are not essential for the antihypertensive action. The acute haemodynamic effect of β -adrenoceptor antagonism is a fall in cardiac output (Lysbo Svendsen *et al.*, 1979), and because of baroreflex-mediated compensatory mechanisms, total peripheral vascular resistance initially rises (Lund-Johansen, 1979). The long-term effect, however, is a reduction of arterial resistance associated with return of cardiac output towards baseline values. Therefore, it appears that β -blockers lower blood pressure by influencing the regulation of vascular tone, which leads to vasodilatation in the resistance vasculature (Man in't Veld, 1991).

The mechanism of the vasodilatation during β -adrenoceptor antagonism is not fully understood. Interference with

vasoconstrictor nerve activity through blockade of prejunctional β -adrenoceptors with subsequent inhibition of noradrenaline (NA) release could lead to diminished arterial contractility, and thus explain the antihypertensive effect (Meiracker *et al.*, 1989). In addition, endothelial influences may play a role, since arterial preparations of normotensive animals have been shown to relax in the presence of β -blockers if the endothelium is left intact (Mostaghim *et al.*, 1986; Janczewski *et al.*, 1987). Moreover, treatment with atenolol has been reported to stimulate vasodepressor prostaglandin generation in the kidney and aorta of hypertensive rats (Hirawa *et al.*, 1991).

Since the long-term effects of β -blockade on arterial function are largely unknown, the aim of the present study was to elucidate the influences of treatment with atenolol, a hydrophilic β_1 -selective compound, on vascular responses in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. We examined arterial contractions induced by membrane depolarization and α -adrenoceptors, and relaxations to endothelium-dependent and -independent agents after the atenolol therapy. In addition, vascular smooth muscle ability to sequester calcium into cellular stores and to induce relaxation via Na⁺,K⁺-ATPase were evaluated by indirect methods (Kojima *et al.*, 1991; Arvola *et al.*, 1992).

¹ Author for correspondence.

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 8 weeks of age both SHR and WKY rats were divided into two groups of equal mean systolic blood pressures. Thereafter, SHR ($n = 10$) and WKY rats ($n = 10$) were given atenolol in drinking water in light-proof bottles, (average dose $25 \text{ mg kg}^{-1} \text{ day}^{-1}$, fresh drug solutions daily prepared), while untreated SHR ($n = 15$) and normotensive WKY rats ($n = 15$) were kept on normal drinking fluid. This daily dose of the drug was chosen, since in the range $15\text{--}45 \text{ mg kg}^{-1}$ atenolol has been reported to induce a comparable and nearly maximal affect on blood pressure in SHR (Takeda *et al.*, 1982). The concentration of atenolol in the drinking water was adjusted weekly to the drinking habits of the animals. Atenolol therapy and indirect blood pressure measurements continued for 14 more weeks until the animals were 22 weeks old. Thereafter atenolol administration was withdrawn 1 day before the rats were decapitated and exsanguinated. The blood samples were drawn into polystyrene tubes containing heparin ($100 \text{ units ml}^{-1}$) as anticoagulant for lymphocyte intracellular free calcium concentration ($[\text{Ca}^{2+}]_i$) measurements, which were performed as previously described (Pörsti *et al.*, 1992; Wuorela *et al.*, 1992). 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 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. The most distal ring was left with the endothelium intact and from the other three vascular endothelium was removed by gently rubbing with a jagged injection needle (Arvola *et al.*, 1992). The average medial thickness in this preparation is about 110 and $80 \mu\text{m}$ in SHR and WKY rats, respectively (Arvola *et al.*, 1993; Wuorela *et al.*, 1994). The rings were placed between stainless steel hooks (diameter $30 \mu\text{m}$) 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 (FT03 transducer and model 7E 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 $1 \mu\text{M}$ acetylcholine (ACh) in $1 \mu\text{M}$ 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 a 30 min stabilization, the endothelium-denuded mesenteric arterial ring was contracted with $10 \mu\text{M}$ NA to provide a reference contraction. When the maximal response had fully developed, the rings were rinsed

with Ca^{2+} -free PSS, and once the resting tension was restored the contraction to NA was repeated in Ca^{2+} -free medium to deplete the cellular calcium stores. After a further 10 min equilibration in resting tension at 0 mM calcium with 1 mM ethylene glycol *bis*(β -aminoethyl ether)- N,N,N',N' -tetraacetic acid (EGTA), the rings were rinsed several times with Ca^{2+} -free buffer without EGTA and again contracted with NA ($10 \mu\text{M}$, in Ca^{2+} -free PSS). When the response had reached a plateau, calcium was cumulatively readded to the organ bath in concentrations of 0.05, 0.1, 0.2, 0.4, 0.8, 1.2 and 2.5 mM, and the increase in contractile force registered. After the maximal response, rings were rinsed with normal PSS and allowed a 20 min recovery period. Then the cycle was repeated with 125 mM KCl used as the agonist instead of NA. In solutions containing high concentrations of potassium, NaCl was substituted with KCl on an equimolar basis.

Vascular preparation 2 After a 30 min stabilization period, the endothelium-denuded vascular ring was contracted with $10 \mu\text{M}$ NA (reference contraction). When the maximal contraction was reached, the ring was rinsed with Ca^{2+} -free PSS. After a 10-min period in Ca^{2+} -free PSS, 1 mM EGTA was introduced and maximal response with $10 \mu\text{M}$ NA was elicited 1 min later. This induced rapidly fading contraction, the peak of the response reflecting the amount of calcium liberated from cellular stores (Dohi *et al.*, 1990). The following cycle was then used to evaluate calcium sequestration into cellular stores: (1) The preparations were incubated for 6 min in Ca^{2+} -free PSS containing 1 mM EGTA (depletion of cellular stores). (2) The rings were allowed a 10-min recovery period in PSS with calcium (calcium loading period; no EGTA in organ bath). (3) The Ca^{2+} -free PSS containing 0.1 mM EGTA was returned to the organ bath and 1 min later contractions to $10 \mu\text{M}$ NA were again elicited and maximal responses registered. This cycle was repeated 5 times, the following organ bath calcium concentrations during the calcium loading period being tested: 0, 0.033, 0.1, 0.4 and 1.2 mM. After a 20-min recovery period similar cycles were repeated with 30 mM caffeine as the contractile agent.

Vascular preparation 3 After 30 min the endothelium-denuded preparation was contracted with 125 mM KCl (reference response). 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. Once the contraction had reached a plateau, 1 mM potassium was re-added and the subsequent relaxation registered.

Vascular preparation 4 In the most distal ring with intact vascular endothelium, concentration-response curves for NA, and 30 min later for KCl, were determined cumulatively. After another 30 min recovery period, vascular responses to ACh, 3-morpholinylsydnominine (SIN-1), and isoprenaline were examined. The rings were precontracted with $1 \mu\text{M}$ NA, and after the contraction had developed fully increasing concentrations of the relaxing agent were cumulatively added to the organ bath. The next concentration of the relaxant was added only after the previous level of relaxation was stable. The rings were allowed a 20-min recovery in resting tension between the study of each relaxant.

The KCl-, NA- and K^+ -free medium-induced contractile responses were expressed in g. The EC_{50} for KCl and NA in each ring was calculated as percentage of maximal response. Calcium cumulative responses in Ca^{2+} -free buffer solution with KCl and NA as the agonists, and the maximal contractile forces induced by 30 mM caffeine and $10 \mu\text{M}$ NA after each calcium loading period were normalized by relating them to the previously determined reference contractions. After potassium repletion upon K^+ -free contractions, the greatest reduction in smooth muscle contractile force during

a 1 min period was considered the maximal relaxation rate, the mean relaxation rate being calculated from the time which was required to reach the maximal level of potassium relaxation. The relaxations in response to potassium repletion, ACh, SIN-1 and isoprenaline were presented as percentage of pre-existing contractile force. The EC_{50} for the latter 3 relaxants was calculated as a percentage of the $1 \mu M$ NA-induced precontraction. All EC_{50} values were calculated with a computer programme and presented as the negative logarithm (pD_{50}), which values were also used in the statistical analysis.

Drugs

The following drugs were used: acetylcholine chloride, calcium salt of ionomycin, EGTA, isoprenaline hydrochloride, ammonium salt of heparin (Sigma Chemical Co., St. Louis, Mo., U.S.A.), atenolol (Leiras Pharmaceutical Co., Turku, Finland), acetoxymethyl ester of quin-2, Triton X100 (Aldrich Chemical Co., Milwaukee, Wis., U.S.A.), 3-morpholinolinosynonimine (GEA Ltd., Copenhagen, Denmark) and (-)-noradrenaline L-hydrogentartrate (Fluka Chemie AG, Buchs SG, Switzerland). Atenolol 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) and quin-2 (in dimethylsulphoxide). 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 Bonferroni confidence intervals 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-group differences. Differences were considered significant when $P < 0.05$. All results are expressed as mean $\pm$ s.e.mean.

Results

Blood pressure, heart rate, heart and body weights, and intracellular free calcium

The systolic blood pressure of SHR was higher at the beginning of the study than in WKY rats, and during the 14-week-long follow up it increased steadily in both SHR groups,

whereas no significant change was observed in either of the WKY groups. Furthermore, the rise in blood pressure was significantly attenuated in atenolol-treated SHR (Figure 1 and Table 1). Heart rate was also reduced by atenolol administration in SHR, and it remained lower throughout the study than in control SHR (Table 1).

The atenolol therapy did not affect heart or body weights in either strain (Table 1). Lymphocyte $[Ca^{2+}]_i$, measured by the fluorescent indicator quin-2, was comparable in control and atenolol-treated SHR, and markedly higher than in the WKY groups (Table 1).

Mesenteric arterial responses in vitro

The relaxations of endothelium-intact mesenteric arterial rings to ACh were greatly impaired in untreated SHR when compared with the WKY groups. Interestingly, the response to ACh was markedly improved in SHR by the atenolol treatment, the relaxation not differing from that in the WKY groups. Moreover, following maximal dilatations to ACh the force of contraction rapidly increased in untreated SHR, but these reconstrictions were practically absent in WKY rats and

Figure 1 Systolic blood pressure in untreated spontaneously hypertensive rats (SHR, $\circ$), atenolol-treated SHR ($\bullet$, $25 \text{ mg kg}^{-1} \text{ day}^{-1}$), untreated Wistar-Kyoto (WKY, $\square$) rats, and atenolol-treated WKY rats ($\blacksquare$). Symbols indicate means with s.e.means, $n = 10-12$ in each group; $*P < 0.05$, ANOVA for repeated measurements.

Table 1 Blood pressure, body weight and heart rate during the study, and heart weight and lymphocyte intracellular free calcium concentration at the close of the 13-week study

Variable	SHR	Aten-SHR	WKY	Aten-WKY
Systolic blood pressure (mmHg)				
week 0	$165 \pm 4^*$	$166 \pm 3^*$	131 ± 5	133 ± 3
week 14	$223 \pm 3^*$	$191 \pm 4^{*†}$	143 ± 3	140 ± 4
Body weight (g)				
week 0	197 ± 6	199 ± 4	200 ± 9	205 ± 8
week 14	374 ± 4	368 ± 6	379 ± 7	370 ± 8
Heart weight (g kg^{-1} body weight)	$3.6 \pm 0.1^*$	$3.6 \pm 0.1^*$	3.2 ± 0.1	3.1 ± 0.1
Heart rate (beats min^{-1})				
week 0	$374 \pm 4^*$	$375 \pm 3^*$	328 ± 5	330 ± 4
week 14	$335 \pm 8^*$	$302 \pm 3^{*†}$	289 ± 5	285 ± 6
Intracellular free calcium concentration in lymphocytes (nM)	$98 \pm 3^*$	$99 \pm 6^*$	72 ± 3	74 ± 4

Values are mean $\pm$ s.e.mean; $n = 10-12$ in each group. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; Aten, atenolol-treated rats. The 14-week long atenolol treatment commenced at study week 0 when the animals were 8 weeks old. $*P < 0.05$ compared with WKY rats, $†P < 0.05$ Aten-SHR versus SHR (Bonferroni test).

atenolol-treated SHR (Table 2 and Figure 2). No differences were found in the nitric oxide donor SIN-1-induced responses between the study groups (Figure 2). However, the relaxation to isoprenaline was attenuated in untreated SHR when compared with WKY rats. The atenolol therapy also improved this response in SHR, but the relaxation nevertheless remained less marked than in WKY rats (Table 2 and Figure 3).

The maximal contractions elicited by K^+ -free solution did not differ between the four study groups (Table 3). After the return of potassium to the organ bath upon K^+ -free contractions the rate of the subsequent relaxation was faster in the two WKY groups than in SHR. Furthermore, the atenolol therapy clearly enhanced the rate of potassium relaxation in SHR (Figure 3). However, the time to reach baseline tension after washout of 125 mM KCl, i.e. the washout time, which was clearly prolonged in SHR when compared with WKY rats, was not shortened by the atenolol treatment in SHR (Table 3).

In endothelium-denuded mesenteric arterial rings maximal contractile force generation to high concentrations of KCl was more pronounced in SHR than in WKY rats, and these responses were not affected by the atenolol treatment in either strain. The contractions to NA, however, were comparable in untreated SHR and WKY rats, while atenolol slightly increased the maximal response in SHR but not in WKY rats (Figure 4). pD_{50} values of KCl- and NA-induced contractions were corresponding in all study groups (Table 2).

The effect of organ bath calcium concentration on 125 mM KCl- and 10 μ M NA-induced contractions, i.e. the calcium sensitivity of the vascular rings during depolarization and α -adrenoceptor stimulation, respectively, were comparable in all four study groups (Figure 5).

The contractile responses induced by 30 mM caffeine and

Figure 2 Relaxations of endothelium-intact mesenteric arterial rings after precontraction with 1 μ M noradrenaline from untreated spontaneously hypertensive rats (SHR, $\circ$), atenolol-treated SHR ($\bullet$), untreated Wistar-Kyoto (WKY, $\square$) rats, and atenolol-treated WKY rats ($\blacksquare$). The relaxation to acetylcholine consisted of the maximal response (a) and the subsequent recontraction (b). Relaxations were also induced by 3-morpholinosydnonimine (SIN-1) (c). Symbols indicate means with s.e.mean, $n = 8$ in each group; * $P < 0.05$, ANOVA for repeated measurements.

Table 2 Parameters of contractile and relaxation responses of isolated endothelium-intact arterial rings

Variable	SHR	Aten-SHR	WKY	Aten-WKY
Relaxation responses				
pD_{50}				
Acetylcholine	$7.20 \pm 0.20^*$	$7.70 \pm 0.05^\dagger$	7.79 ± 0.05	7.64 ± 0.07
SIN-1	6.34 ± 0.11	6.53 ± 0.10	6.38 ± 0.03	6.54 ± 0.11
Isoprenaline	$4.89 \pm 0.23^*$	$5.22 \pm 0.10^*$	5.83 ± 0.22	5.70 ± 0.25
Maximal relaxation				
(% of 1 μ M noradrenaline-induced precontraction)				
Acetylcholine (10 μ M)	$87 \pm 3^*$	$97 \pm 1^\dagger$	98 ± 1	96 ± 1
SIN-1 (3.3 μ M)	91 ± 3	96 ± 2	95 ± 2	98 ± 2
Isoprenaline (0.1 mM)	$63 \pm 5^*$	$76 \pm 1^{\dagger*}$	84 ± 2	85 ± 3
Contractile responses				
pD_{50}				
Noradrenaline	6.33 ± 0.04	6.33 ± 0.02	6.37 ± 0.08	6.45 ± 0.08
Potassium chloride	1.32 ± 0.01	1.30 ± 0.02	1.36 ± 0.03	1.37 ± 0.03
Maximal force (g)				
Noradrenaline (10 μ M)	3.0 ± 0.2	$3.5 \pm 0.2^{\dagger*}$	2.9 ± 0.2	3.1 ± 0.2
Potassium chloride (125 mM)	$3.3 \pm 0.1^*$	$3.5 \pm 0.1^*$	2.6 ± 0.2	2.5 ± 0.2

Values are mean $\pm$ s.e.mean; $n = 10$ –12 in each group. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; Aten, atenolol-treated rats. EC_{50} values are presented at the negative logarithm (pD_{50}) of concentration of the agonist. * $P < 0.05$ compared with WKY rats, $^\dagger P < 0.05$ Aten-SHR versus SHR (Bonferroni test).

10 μ M NA after loading periods in increasing organ bath calcium concentrations were clearly less marked in SHR than in WKY rats. These contractions, reflecting the ability of

arterial smooth muscle to sequester calcium into cellular stores, were improved in SHR by the atenolol treatment (Figure 6).

Figure 3 Relaxations to isoprenaline after precontraction with 1 μ M noradrenaline in endothelium-intact mesenteric arterial rings (a), and relaxations to re-addition of 1.0 mM K^+ after precontraction induced by K^+ -free buffer solution in endothelium-denuded rings (b). The groups were untreated spontaneously hypertensive rats (SHR, $\circ$), atenolol-treated SHR ($\bullet$), untreated Wistar-Kyoto (WKY, $\square$) rats, and atenolol-treated WKY rats ($\blacksquare$). Symbols indicate means with s.e.mean, $n = 8-10$ in each group; * $P < 0.05$, ANOVA for repeated measurements.

Figure 4 Concentration-response curves of endothelium-denuded mesenteric arterial rings to potassium chloride (a) and noradrenaline (b) in untreated spontaneously hypertensive rats (SHR, $\circ$), atenolol-treated SHR ($\bullet$), untreated Wistar-Kyoto (WKY, $\square$) rats, and atenolol-treated WKY rats ($\blacksquare$). Contractile force generation to potassium chloride was more pronounced in SHR than WKY rats, and atenolol treatment slightly increased maximal responses to noradrenaline in SHR. Symbols indicate means with s.e.mean, $n = 8$ in each group; * $P < 0.05$, one-way ANOVA.

Table 3 Parameters of potassium-free buffer solution-induced contractions and subsequent relaxations induced by 1.0 mM potassium chloride in isolated endothelium-denuded mesenteric arterial rings

Variable	SHR	Aten-SHR	WKY	Aten-WKY
Reference response to 125 mM potassium chloride				
Maximal force (g)	2.3 ± 0.1	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.2
Washout time (min)	$16.0 \pm 1.0^*$	$13.3 \pm 0.8^*$	7.7 ± 0.6	7.5 ± 0.4
Potassium-free contraction				
Time to onset (min)	3.0 ± 0.5	3.3 ± 0.6	5.1 ± 1.3	4.8 ± 0.4
Maximal force (g)	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1
Potassium-relaxation				
Maximal rate (mg min^{-1})	$75 \pm 13^*$	$245 \pm 31^\dagger$	317 ± 64	330 ± 80
Mean rate (mg min^{-1})	$51 \pm 8^*$	$113 \pm 10^\dagger$	111 ± 17	121 ± 14

Values are mean $\pm$ s.e.mean; $n = 10-12$ in each group. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; Aten, atenolol-treated rats. * $P < 0.05$ compared with WKY rats, $^\dagger P < 0.05$ Aten-SHR versus SHR (Bonferroni test).

Figure 5 Contractile responses of endothelium-denuded mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR, ○), atenolol-treated SHR (●), untreated Wistar-Kyoto rats (WKY, □) and, atenolol-treated WKY rats (■). Line graphs show the effect of organ bath calcium concentration on 125 mM KCl- (a), and 10 μ M noradrenaline-induced contractions (b). Symbols indicate means with s.e.mean, $n = 8-10$ in each group.

Discussion

In the present study, the atenolol therapy had a moderate antihypertensive action in SHR, whereas blood pressure in WKY rats was not affected. However, heart weights were not decreased by the treatment in SHR. Apparently the reduction in afterload accompanying the moderately lowered blood pressure was not sufficient to attenuate cardiac hypertrophy. Moreover, the degree of cardiovascular hypertrophy in SHR is not only governed by the level of blood pressure, but also by enhanced cellular responses to various growth factors such as angiotensin II (Dzau *et al.*, 1991). Previous reports have shown either no change (Chatelain *et al.*, 1981) or an attenuated increase (Lauva & Tomanek, 1985) in heart weights after chronic β_1 -adrenoceptor blockade in SHR.

ACh relaxes arteries endothelium-dependently via the release of the endothelium-derived relaxing factor (EDRF), which stimulates smooth muscle soluble guanylate cyclase and elevates intracellular guanosine 3':5'-cyclic monophosphate (cyclic GMP) (Moncada *et al.*, 1991). EDRF is probably identical with nitric oxide (NO) (Moncada *et al.*, 1991), the production of which is also the mechanism of action of the endothelium-independent vasodilator, SIN-1 (Feelisch *et al.*, 1989). Previously several studies have reported impaired endothelium-dependent relaxation in human and experimental

Figure 6 Contractile responses reflecting the ability to sequester calcium into cellular stores in endothelium-denuded mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR, ○), atenolol-treated SHR (●), untreated Wistar-Kyoto (WKY, □) rats, and atenolol-treated WKY rats (■). Initially, cellular calcium stores were depleted, whereafter calcium was returned to the organ bath for 10 min, the concentrations 0, 0.033, 0.1, 0.4 and 1.2 mM being tested. The contractions induced by 30 mM caffeine (a) and 10 μ M noradrenaline (b) after each calcium loading period were normalized by comparing them to the previously determined reference responses. Symbols indicate means with s.e.means, $n = 8-10$ in each group; * $P < 0.05$, ANOVA for repeated measurements.

forms of hypertension (Watt & Thurston, 1989; Treasure *et al.*, 1991; Arvola *et al.*, 1993), a finding which was confirmed in the present study. Interestingly, the moderate antihypertensive effect of the atenolol treatment was accompanied by normalization of the response to ACh in SHR. However, no significant differences in the relaxations elicited by SIN-1 were found between the study groups. Thus, the sensitivity of arterial smooth muscle to NO was comparable in both hypertensive and normotensive rats, and was not altered by β -adrenoceptor antagonism. Furthermore, arterial relaxation elicited by isoprenaline has in general been considered to be mediated endothelium-independently via β -adrenoceptor stimulation and the subsequent increase in intracellular adenosine 3':5'-cyclic monophosphate (cyclic AMP) in smooth muscle (Bülbring & Tomita, 1987). However, the endothelium, too, contains β -adrenoceptors (Steinberg *et al.*, 1984), the activation of which increases cyclic AMP within the endothelial cells, which, in turn, augments the release of NO and promotes vascular relaxation (Gray & Marshall, 1992; Graves & Poston, 1993). Since β -adrenoceptor agonists

thus are partially endothelium-dependent vasodilators, this mechanism may have contributed to the augmented relaxation to isoprenaline observed in atenolol-treated SHR. Upregulation of β -adrenoceptors in smooth muscle remains another possibility to explain the improved isoprenaline response (Meiracker *et al.*, 1989). Taken together, the normalized response to ACh together with enhanced relaxation to isoprenaline in SHR following the atenolol therapy suggest improved endothelial function in these animals.

Previously, treatment with angiotensin converting enzyme inhibitors has been shown to normalize the relaxation to ACh in SHR (Clozel *et al.*, 1990; Arvola *et al.*, 1993), whereas diuretic therapy with trichlormethiazide only moderately augmented the response (Kähönen *et al.*, unpublished observations), and hydralazine treatment was without effect on the endothelium-dependent relaxation (Clozel *et al.*, 1990). Thus, although all of these antihypertensive therapies reduced blood pressure, their effects on endothelium-dependent relaxation appeared diverse. Therefore, the enhanced ACh-induced relaxation after the long-term atenolol treatment may not be related exclusively to the reduction of blood pressure but to other mechanisms affecting endothelial function as well.

After maximal relaxations to ACh, marked recontractions developed in untreated SHR but not in WKY rats and atenolol-treated SHR. ACh is known to evoke contractions in blood vessels of SHR but not of WKY rats via the release of endothelium-derived contractile factors (EDCF) (Lüscher & Vanhoutte, 1986). Furthermore, the production of EDCF appears to parallel closely the increase in blood pressure (Iwama *et al.*, 1992), and the impaired dilator response to ACh in SHR probably does not result from deficient NO production, but from enhanced simultaneous release of contractile factors from the endothelium (Lüscher & Vanhoutte, 1986; Ito & Carretero, 1992; Auch-Schweik *et al.*, 1992). Thus, in the present study the absence of the recontractions after maximal relaxations to ACh in the treated SHR suggests that the release of contractile factors from the endothelium was diminished by the atenolol therapy, the mechanism of which may also underlie the improved relaxations to ACh. This conclusion is further supported by the fact that no significant differences in the relaxations to SIN-1 were detected in the study groups. Hence, the balance between endothelium-dependent relaxation and contraction may be beneficially modified by atenolol. Previous investigations have shown that several β -blockers can relax arterial preparations from normotensive animals, and that this effect is more pronounced in the presence of an intact endothelium (Mostaghim *et al.*, 1986). Moreover, carteolol has been reported to augment the basal release of relaxing factors from the endothelium of canine femoral artery (Janczewski *et al.*, 1987), and nebivolol to induce endothelium-dependent relaxation of canine coronary artery (Gao *et al.*, 1991), while celiprolol has neither direct nor indirect relaxing effects in rat arteries and veins (O'Rourke & Vanhoutte, 1990). Therefore, further investigations are clearly needed to clarify the acute and chronic effects of β -adrenoceptor blockade on endothelial function in hypertension.

Vascular contractions induced by Na^+ , K^+ -ATPase inhibition with a K^+ -free medium in arterial rings result from sodium leak to the smooth muscle cells, which causes depolarization and increases calcium influx through voltage-dependent channels (Mulvany, 1985). In addition, the depolarization of vascular adrenergic nerve-endings releases NA, which contributes to the contraction (Vanhoutte & Lorenz, 1984). In the present study, vascular Na^+ , K^+ -ATPase function was evaluated indirectly by readdition of potassium upon full K^+ -free medium-induced precontractions (Arvola *et al.*, 1992). The return of potassium activates Na^+ , K^+ -ATP which repolarizes the cell membrane and initiates the relaxation of smooth muscle (Bonaccorsi *et al.*, 1977). The potassium relaxation rate was markedly slower in SHR than WKY rats, in agreement with earlier observations

(Arvola *et al.*, 1992; Pörsti *et al.*, 1992), and was clearly enhanced in SHR after the atenolol therapy. However, the washout time after maximal contractions to KCl was not significantly shortened by atenolol. The markedly prolonged interval required to reach baseline tension after washout of 125 mM KCl in SHR presumably reflects abnormal excitation-contraction uncoupling mechanisms with impaired sequestration and extrusion of intracellular calcium, and dephosphorylation of contractile proteins. Therefore, the enhanced potassium relaxation indicates promoted recovery rate of ionic gradients across the cell membrane in SHR following long-term β -adrenoceptor blockade, probably via improved function of vascular Na^+ , K^+ -ATPase.

In the study described here, the ability of arterial smooth muscle to sequester calcium was examined by means of caffeine- and NA-induced contractions after loading periods in different organ bath calcium concentrations. Of these two agonists, caffeine directly (see Karaki & Weiss, 1988) and NA via the stimulation of 1,4,5-inositol triphosphate accumulation (Guild *et al.*, 1992; Itoh *et al.*, 1992) opens the calcium channels in the sarcoplasmic reticulum. The refilling of the calcium stores without cell activation and $[\text{Ca}^{2+}]_i$ elevation presumably occurs via a direct link between the extracellular space and intracellular calcium stores (Rink, 1990). After the calcium loading periods the contractions to both NA and caffeine were lower in SHR than WKY rats, in agreement with previous reports (Dohi *et al.*, 1990; Wuorela *et al.*, 1994), and atenolol treatment improved these responses in SHR. Thus, the present results suggest that the ability of the sarcoplasmic reticulum to take up and store NA- and caffeine-releasable calcium is improved in SHR by atenolol treatment.

The concentration of intracellular free calcium is a primary determinant of arterial tone (Rembold, 1992). Previously, abnormalities in calcium metabolism have been reported to be prior events rather than subsequent to the development of hypertension in SHR, since elevated $[\text{Ca}^{2+}]_i$ has been found in vascular smooth muscle of prehypertensive animals (Sugiyama *et al.*, 1990), and in circulating as well as in non-circulating cells (Oshima *et al.*, 1991; Pörsti *et al.*, 1992). Moreover, normalization of blood pressure and arterial relaxation in SHR by ACE-inhibitor therapy has been reported to normalize the elevated $[\text{Ca}^{2+}]_i$ in platelets and lymphocytes of these animals (Arvola *et al.*, 1993). In the present study, basal $[\text{Ca}^{2+}]_i$ was clearly higher in lymphocytes of SHR when compared with WKY rats, and was not reduced by atenolol therapy in SHR. Thus, assuming that the observed changes in lymphocytes $[\text{Ca}^{2+}]_i$ also reflected cellular calcium metabolism in other tissues, the reduction in blood pressure following long-term β -adrenoceptor blockade was not associated with reduced basal cytosolic calcium in SHR. However, the above results on the ability of sarcoplasmic reticulum to take up and store calcium in smooth muscle suggest that the atenolol therapy had a beneficial effect on cellular calcium handling even though the basal calcium concentration was not reduced.

Arterial contractions to the classical contractile agents KCl and NA, and vascular calcium sensitivity during stimulation with these agonists were also examined. KCl-induced contractions were not affected by atenolol therapy in the two strains, and also the sensitivity to NA remained unchanged. The maximal contractile force generation to NA, however, was slightly increased by atenolol in SHR but not in WKY rats. The explanation for this remains unclear, since any residual β -adrenoceptor blockade would be expected to produce increased maximal contractions and sensitivity to NA in both rat strains. Furthermore, when inducing contractions to NA in a Ca^{2+} -free medium, the response primarily reflects calcium release from the sarcoplasmic reticulum, a minor source of the activator calcium being the inner surface of the plasmalemma, whereas the response to KCl depends mainly upon extracellular calcium (Johns *et al.*, 1987). Thus, the increase in contractile force during cumulative calcium addition to the organ bath results from calcium entry through the

plasmalemma (Karaki & Weiss, 1988). In the present study, the response to calcium addition with KCl (125 mM) or NA (10 μ M) as the agonists did not significantly differ in SHR and WKY rats and was not affected by the atenolol therapy in either strain. Therefore, long-term β -adrenoceptor blockade did not appear to change arterial calcium sensitivity. In addition, the present findings on contractile responses further support the conclusion that alterations in endothelial function and not in contractile sensitivity or total calcium entry to the smooth muscle explain the augmented arterial relaxation in atenolol-treated SHR. Finally, the beneficial changes in calcium sequestration ability in arterial smooth muscle of atenolol-treated SHR appear to correlate with alterations in relaxation but not in contractile responses. This finding is in agreement with the fact that inhibitors of sarcoplasmic reticulum Ca^{2+} -ATPase also inhibit the relaxation of arterial smooth muscle (Luo *et al.*, 1993).

In conclusion, the moderate antihypertensive effect of atenolol in SHR was accompanied by enhancement of β -

adrenergic and normalization of endothelium-dependent arterial relaxation. Furthermore, the responses reflecting the ability to sequester calcium into cellular stores and to induce vascular relaxation via function of the Na^+, K^+ -ATPase were augmented. In contrast, the contractions to KCl and NA, as well as vascular calcium sensitivity, remained practically unaffected after the chronic β -adrenoceptor blockade. Therefore, the present results suggest that enhanced arterial relaxation and improved smooth muscle calcium handling participate in the long-term blood pressure-lowering action of atenolol in this type of genetic hypertension.

This study was supported by the Finnish Cultural Foundation, Häme Fund (M.K.) and the Paavo Nurmi Foundation, Finland (I.P.). Atenolol was kindly donated by Leiras Pharmaceutical Company, Turku, Finland. The valuable discussions with Dr Kazuhide Ayajiki are sincerely acknowledged.

References

- ARVOLA, P., PÖRSTI, I., VUORINEN, P., PEKKI, A. & VAPAATALO, H. (1992). Contractions induced by potassium-free solution and potassium relaxation in vascular smooth muscle of hypertensive and normotensive rats. *Br. J. Pharmacol.*, **106**, 157–165.
- ARVOLA, P., RUSKOAHO, H., WUORELA, H., PEKKI, A., VAPAATALO, H. & PÖRSTI, I. (1993). Quinapril treatment and arterial smooth muscle responses in spontaneously hypertensive rats. *Br. J. Pharmacol.*, **108**, 980–990.
- AUCH-SCHWELK, W., KATUSIC, Z.S. & VANHOUTTE, P.M. (1992). Nitric oxide inactivates endothelium-derived contracting factor in the rat aorta. *Hypertension*, **19**, 442–445.
- BÜLBRING, E. & TOMITA, T. (1987). Catecholamine action on smooth muscle. *Pharmacol. Rev.*, **39**, 49–96.
- BONACCORSI, A., HERMSMEYER, K., APRIGLIANO, O., SMITH, C.B. & BOHR, D.F. (1977). Mechanisms of potassium relaxation of arterial muscle. *Blood Vessels*, **14**, 261–276.
- CHATELAIN, P., WAELEBROECK, M., CAMUS, J.-C., DE NEEF, P., ROBERECHT, P., ROBA, J. & CHRISTOPHE, J. (1981). Comparative effects of α -methyldopa, propranolol and hydralazine therapy on cardiac adenylate cyclase activity in normal and spontaneously hypertensive rats. *Eur. J. Pharmacol.*, **72**, 17–25.
- CLOZEL, M., KUHN, H. & HEFTI, J. (1990). Effects of angiotensin converting enzyme inhibitors and of hydralazine on endothelial function in hypertensive rats. *Hypertension*, **16**, 532–540.
- DOHI, Y., AOKI, K., FUJIMOTO, S., KOJIMA, M. & MATSUDA, T. (1990). Alteration in sarcoplasmic reticulum-dependent contraction of tail arteries in response to caffeine and noradrenaline in spontaneously hypertensive rats. *J. Hypertens.*, **8**, 261–267.
- DZAU, V.J., GIBBONS, G.H. & PRATT, R.E. (1991). Molecular mechanisms of vascular renin-angiotensin system in myointimal hyperplasia. *Hypertension*, **18**, (suppl. II), II-100–II-105.
- FEELISCH, M., OSTROWSKI, J. & NOACK, E. (1989). On the mechanism of NO release from sydnonimines. *J. Cardiovasc. Pharmacol.*, **14**, (suppl. 11), S13–S22.
- GAO, Y., NAGAO, T., BOND, R.A., JANSSENS, W.J. & VANHOUTTE, P.M. (1991). Nebivolol induces endothelium-dependent relaxations of canine coronary arteries. *J. Cardiovasc. Pharmacol.*, **17**, 964–969.
- GRAVES, J. & POSTON, L. (1993). β -Adrenoceptor agonist mediated relaxation of rat isolated resistance arteries: a role for the endothelium and nitric oxide. *Br. J. Pharmacol.*, **108**, 631–637.
- GRAY, D.W. & MARSHALL, I. (1992). Novel signal transduction pathway mediating endothelium-dependent β -adrenoceptor vasorelaxation in rat thoracic aorta. *Br. J. Pharmacol.*, **107**, 684–690.
- GUILD, S.B., JENKINSON, S. & MUIR, T.C. (1992). Noradrenaline-stimulated inositol phosphate accumulation in arteries from spontaneously hypertensive rats. *Br. J. Pharmacol.*, **106**, 859–864.
- HIRAWA, N., UEHURA, Y., NUMABE, A., TAKADA, S., MATSUOKA, H., IKEDA, T., SUGIMOTO, T., YAGI, S. & ISHII, M. (1991). Stimulating effects of atenolol on vasodepressor prostaglandin generation in spontaneously hypertensive rats. *Clin. Sci.*, **81**, 499–507.
- ITO, S. & CARRETERO, O.A. (1992). Impaired response to acetylcholine despite intact endothelium-derived relaxing factor/nitric oxide in isolated microperfused afferent arterioles of the spontaneously hypertensive rats. *J. Cardiovasc. Pharmacol.*, **20**, (suppl. 12), S187–S189.
- ITOH, T., KAJIKURI, J. & KURIYAMA, H. (1992). Characteristic features of noradrenaline-induced Ca^{2+} mobilization and tension in arterial smooth muscle of the rabbit. *J. Physiol.*, **457**, 297–314.
- IWAKA, Y., KATO, T., MURAMATSU, M., ASANO, H., SHIMIZU, K., TOKI, Y., MIYAZAKI, Y., OKUMURA, K., HASHIMOTO, H., ITO, T. & SATAKE, T. (1992). Correlation with blood pressure of the acetylcholine-induced endothelium-derived contracting factor in the rat aorta. *Hypertension*, **19**, 326–332.
- JANCZEWSKI, P., BOULANGER, C., IQBAL, A. & VANHOUTTE, P.M. (1987). Endothelium-dependent effects of carteolol. *J. Pharmacol. Exp. Ther.*, **247**, 590–595.
- JOHNS, A., LEIJTEN, P., YAMAMOTO, H., HWANG, K. & VAN BREE-MEN, C. (1987). Calcium regulation in vascular smooth muscle contractility. *Am. J. Cardiol.*, **59**, 18A–23A.
- KARAKI, H. & WEISS, G.B. (1988). Calcium release in smooth muscle. *Life Sci.*, **42**, 111–122.
- KOJIMA, M., AOKI, K., ASANO, M., FUJIMOTO, S. & MATSUDA, T. (1991). Malfunction of arterial sarcoplasmic reticulum leading to faster and greater contraction induced by high-potassium depolarization in young spontaneously hypertensive rats. *J. Hypertens.*, **9**, 783–788.
- LAUVA, I.K. & TOMANEK, R.J. (1985). Left ventricular performance in spontaneously hypertensive rats after chronic β_1 -adrenoceptor blockade with atenolol. *J. Cardiovasc. Pharmacol.*, **7**, 232–237.
- LUND-JOHANSEN, P. (1979). Hemodynamic consequences of long-term beta-blocker therapy: A 5-year follow-up study of atenolol. *J. Cardiovasc. Pharmacol.*, **1**, 487–495.
- LUO, D.-L., NAKAZAWA, M., ISHIBASHI, T., KATO, K. & IMAI, S. (1993). Putative, selective inhibitors of sarcoplasmic reticulum Ca^{2+} -pump ATPase inhibit relaxation by nitroglycerin and atrial natriuretic factor of the rabbit aorta contracted by phenylephrine. *J. Pharmacol. Exp. Ther.*, **265**, 1187–1192.
- LÜSCHER, T.F. & VANHOUTTE, P.M. (1986). Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension*, **8**, 344–348.
- LYSBO SVENDSEN, T., HARTLING, O. & TRAP-JENSEN, J. (1979). Immediate haemodynamic effects of propranolol, practolol, pindolol, atenolol and ICI 89,406 in healthy volunteers. *Eur. J. Clin. Pharmacol.*, **15**, 223–228.
- MAN IN'T VELD, A.J., MEIRACKER, A. & SCHALEKAMP, M.A.D.H. (1986). The effect of β blockers on total peripheral resistance. *J. Cardiovasc. Pharmacol.*, **8**, (suppl. 4), S49–S60.
- MAN IN'T VELD, A.J. (1991). Vasodilatation, not cardiodepression, underlies the antihypertensive effect of beta-adrenoceptor antagonists. *Am. J. Cardiol.*, **67**, 13B–17B.

- MEIRACKER, A.H., MAN IN'T VELD, A.J., BOOMSMA, F., FISCHBERG, D.J., MOLINOFF, P.B. & SCHALEKAMP, M.A.D.H. (1989). Hemodynamic and β -adrenergic receptor adaptations during long-term β -adrenoceptor blockade: studies with acebutolol, atenolol, pindolol, and propranolol in hypertensive patients. *Circulation*, **80**, 903–914.
- MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.
- MOSTAGHIM, R., MADDOX, Y.T. & RAMWELL, P.W. (1986). Endothelial potentiation of relaxation response to beta adrenoceptor blocking agents. *J. Pharmacol. Exp. Ther.*, **239**, 797–801.
- MULVANY, M.J. (1985). Changes in sodium pump activity and vascular contraction. *J. Hypertens.*, **3**, 429–436.
- O'ROURKE, S.T. & VANHOUTTE, P.M. (1990). Celiprolol has no direct or indirect effects in isolated arteries and veins. *J. Cardiovasc. Pharmacol.*, **15**, 831–835.
- OSHIMA, T., YOUNG, E.W. & MCCARRON, D.A. (1991). Abnormal platelet and lymphocyte calcium handling in prehypertensive rats. *Hypertension*, **18**, 111–115.
- PÖRSTI, I., ARVOLA, P., WUORELA, H. & VAPAATALO, H. (1992). High calcium diet augments vascular potassium relaxation in spontaneously hypertensive rats. *Hypertension*, **19**, 85–92.
- REMBOLD, C.M. (1992). Regulation of contraction and relaxation in arterial smooth muscle. *Hypertension*, **20**, 129–137.
- RINK, T.J. (1990). Receptor-mediated calcium entry. *FEBS Lett.*, **268**, 381–385.
- STEINBERG, S.F., JAFFE, E.A. & BILEZIKIAN, J.P. (1984). Endothelial cells contain beta adrenoceptors. *Naunyn-Schmied. Arch. Pharmacol.*, **325**, 310–313.
- SUGIYAMA, T., YOSHIZUMI, M., TAKAKU, F. & YAZAKI, Y. (1990). Abnormal calcium handling in vascular smooth muscle cells of spontaneously hypertensive rats. *J. Hypertens.*, **8**, 369–375.
- TAKEDA, K., NAKAGAWA, Y., CHIN, W.P. & IMAI, S. (1982). A comparison of antihypertensive effects of atenolol and propranolol in the spontaneously hypertensive, DOCA/saline hypertensive and renal hypertensive rats. *Jpn. J. Pharmacol.*, **32**, 283–289.
- TREASURE, C.B., MANOUKIAN, S.V., KLEIN, J.L., VITA, J.A., NABEL, E.G., RENWICK, G.H., SELWYN, A.P., ALEXANDER, R.W. & GANZ, P. (1992). Epicardial coronary artery responses to acetylcholine are impaired in hypertensive patients. *Circ. Res.*, **71**, 776–781.
- VANHOUTTE, P.M. & LORENZ, R.R. (1984). Na^+ , K^+ -ATPase inhibitors and the adrenergic neuroeffector interaction in the blood vessel wall. *J. Cardiovasc. Pharmacol.*, **6**, (suppl. 1), S88–S94.
- WATT, P.A.C. & THURSTON, H. (1989). Endothelium-dependent relaxation in resistance vessels from the spontaneously hypertensive rats. *J. Hypertens.*, **7**, 661–666.
- WUORELA, H., ARVOLA, P., KÄHÖNEN, M., VAPAATALO, H. & PÖRSTI, I. (1994). Arterial smooth muscle responses in adult and moderately aged spontaneously hypertensive rats. *Pharmacol. Toxicol.*, **74**, 167–173.
- WUORELA, H., PÖRSTI, I., ARVOLA, P., MÄKYNEN, H. & VAPAATALO, H. (1992). Three levels of dietary calcium-effects on blood pressure and electrolyte balance in spontaneously hypertensive rats. *Naunyn-Schmied. Arch. Pharmacol.*, **346**, 524–549.

(Received October 21, 1993

Revised March 14, 1994

Accepted March 28, 1994)