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Effect of celiprolol therapy on arterial dilatation in experimental hypertension

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1 It has recently been suggested that therapy with β-adrenoceptor blockers reduces peripheral arterial resistance via enhanced vascular dilatation. Therefore, we studied the effects of celiprolol, which is a specific β_1-antagonist that has a weak β_2-agonist action, on arterial tone in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats.

2 Two doses of celiprolol (5 and 50 mg kg^{-1} day^{-1}) were administered to the SHR, while the WKY rats received only the higher dose of the drug. During the 12-week treatment period the higher dose attenuated the increase in blood pressure by approximately 20 mmHg in SHR, whereas the lower dose was without significant antihypertensive effect. Celiprolol therapy did not affect blood pressure in the normotensive WKY rats.

3 Responses of mesenteric arterial rings *in vitro* were examined at the end of the study. Interestingly, endothelium-mediated relaxations of noradrenaline (NA)-precontracted rings to acetylcholine (ACh) in the absence and presence of the cyclo-oxygenase inhibitor, diclofenac, were equally enhanced in both celiprolol-treated SHR groups. The nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) practically abolished the relaxations to ACh in all SHR irrespective of whether they had received celiprolol, whereas in WKY rats L-NAME only attenuated the responses to ACh. However, no differences were found between the SHR groups in relaxations to ACh when hyperpolarization of smooth muscle was prevented by precontractions induced by 50 mM KCl. Vasorelaxation of NA-precontracted rings to the exogenous nitric oxide donor, nitroprusside, was also moderately augmented in both celiprolol-treated SHR groups, while the relaxation to β-adrenoceptor agonist, isoprenaline, remained equally impaired in all SHR whether or not they had received celiprolol. No differences were observed between the two WKY groups in the responses to ACh, nitroprusside or isoprenaline.

4 Contractile sensitivity of mesenteric arterial rings to the receptor-mediated agonists, NA and 5-hydroxytryptamine, was comparable in all study groups.

5 In conclusion, SHR treatment with either the low or the higher dose of celiprolol was accompanied by enhancement of both endothelium-dependent and endothelium-independent nitric oxide-mediated arterial relaxation, possibly via a hyperpolarization mechanism. Interestingly, this effect appeared to be independent of the reduction in blood pressure.

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

Introduction

The mechanisms underlying the antihypertensive action of β-adrenoceptor antagonists have not been fully established, but reduction of adrenergic activity, cardiac output and renin release have been proposed to contribute to the lowering of blood pressure (see Man in't Veld *et al.*, 1986). However, it has also been suggested that alterations in these factors are not important, since different β-adrenoceptor antagonists which reduce blood pressure to a comparable extent may have quite dissimilar effects on the above variables (Man in't Veld *et al.*, 1986; Meiracker *et al.*, 1989). 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). Yet, the main haemodynamic change in the long term that leads to the reduction of blood pressure is a decrease in arterial resistance. Therefore, β-adrenoceptor antagonists seem to lower blood pressure by interfering with the regulation of vascular tone which in turn leads to arterial vasodilatation (Meiracker *et al.*, 1988; Man in't Veld, 1991).

The exact mechanism of the vasodilatation during therapy with β-adrenoceptor antagonists is still very much disputed. Several possible explanations have been suggested, including interference with vasoconstrictor nerve activity through blockade of either central, or peripheral prejunctional β-adrenoceptors, enhancement of β-adrenergic and endothelium-dependent arterial relaxation, stimulation of vasodilator prostaglandin generation and normalization of vascular nitric oxide synthase activity (Janczewski *et al.*, 1987; Meiracker *et al.*, 1989; Hirawa *et al.*, 1991; Kähönen *et al.*, 1994; Mehta *et al.*, 1994).

The present study was designed to elucidate the mechanisms underlying the conceivable vasodilator effect of treatment with celiprolol, a hydrophilic β_1-selective adrenoceptor antagonist with a mild selective β_2-adrenoceptor agonist action, by examining vascular responses in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. In particular, the effects of long-term celiprolol therapy on arterial vasodilatation evoked *in vitro* by endothelium-dependent and -independent agents were studied.

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Methods

Animals and experimental design

Male SHR (Okamoto-Aoki strain, $n=36$) and age-matched WKY rats ($n=24$) 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, the SHR were divided into three groups with equal systolic blood pressure and the WKY rats into two groups with equal mean systolic blood pressure. Thereafter, celiprolol was administered in drinking water in light-proof bottles, fresh drug solutions being prepared daily. The drug was given at an average dose of 50 mg kg^{-1} day^{-1} to one group of SHR ($n=12$) and WKY rats ($n=12$), while a second group of SHR ($n=12$) received a lower dose of 5 mg kg^{-1} day^{-1}. Untreated SHR ($n=12$) and WKY rats ($n=12$) were kept on normal drinking fluid. The concentration of celiprolol in the drinking water was weekly adjusted to the drinking habits of the animals. These daily doses of the drug were chosen on the basis of our previous experience of β-adrenoceptor antagonism with atenolol in SHR (Kähönen *et al.*, 1994). Thus, 50 mg kg^{-1} day^{-1} of celiprolol was intended to lower blood pressure significantly in SHR, while 5 mg kg^{-1} day^{-1} was not expected to affect it.

Celiprolol therapy and indirect blood pressure measurements were continued for 12 more weeks until the animals were 20 weeks old. Thereafter, celiprolol administration was withdrawn and 1 day later the rats were anaesthetized by pentobarbitone (35 mg kg^{-1} into the tail vein of conscious animals held in plastic restrainers) and exsanguinated. The hearts were removed and weighed, and the superior mesenteric arteries carefully excised and cleaned of adherent connective tissue. The experimental design of the study was approved by the Animal Experimental Committee of the University of Tampere, Finland.

Mesenteric arterial responses in vitro

Three successive standard sections (3 mm in length) of the mesenteric artery from each animal were cut, beginning 5 mm distally from the mesenteric artery-aorta junction. The rings were placed between stainless steel hooks (diameter 0.3 mm) and suspended in an organ bath chamber (volume 20 ml) in physiological salt solutions (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 30 min 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 7E Polygraph; Grass Instrument Co., Quincy, Ma., U.S.A.). The presence of intact endothelium in vascular preparations was confirmed by a clear relaxation response to 1 μM acetylcholine (ACh) in rings that were pre-contracted with 1 μM noradrenaline (NA).

Arterial relaxation to endothelium-independent and -dependent agents

After the equilibration period, vascular responses to sodium nitroprusside (SNP), isoprenaline and ACh were examined. The rings were pre-contracted with 1 μM NA, and after the contraction had fully developed increasing concentrations of the relaxing agent were added cumulatively to the organ bath. The next concentration of the agonist was added only when the previous level of the response was stable. After the maximal response had been reached, rings were rinsed with PSS and allowed a 20 min recovery period at resting tension. Responses to ACh were then elicited in the presence of 3 μM diclofenac, after this 0.1 mM N^G-nitro-L-arginine methyl ester (L-NAME) was also added to the bath and responses to ACh were re-tested. Finally apamin (1 μM) was added to the bath and the responses were again tested. A further 30 min period was allowed after a new drug was introduced.

Arterial relaxation to endothelium-dependent agents after prevented hyperpolarization

Cumulative relaxation responses to acetylcholine and adenosinediphosphate (ADP) were examined after pre-contractions induced by 50 mM potassium chloride. The responses were then elicited in the presence of 3 μM diclofenac, and after this 0.1 mM L-NAME was also added to the bath and responses to ACh and ADP were re-tested.

Arterial contractions to receptor-mediated agonists

The concentration-response curves for NA and 5-hydroxytryptamine (5-HT) were cumulatively determined. The contractions were then elicited in the presence of 3 μM diclofenac, and after this 0.1 mM L-NAME was also added to the bath and responses to NA and 5-HT were re-tested.

Contractions to NA and 5-HT were presented as a percentage of maximal response. The EC_{50}-values for NA and 5-HT in each ring were calculated as percentage of maximal responses. All EC_{50}-values were calculated with a computer programme and presented as the negative logarithm (pD_2): these values were also used in the statistical analysis. The relaxations to ACh, ADP, SNP and isoprenaline were presented as a percentage of the pre-existing contraction force.

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.

Drugs

The following drugs were used: celiprolol (Leiras Pharmaceutical Co., Turku, Finland), acetylcholine chloride, adenosine diphosphate, apamin, 5-hydroxytryptamine, isoprenaline hydrochloride, N^G-nitro-L-arginine methyl ester hydrochloride (Sigma Chemical Co., St. Louis, Mo., U.S.A.), diclofenac (Ciba-Geigy AG, Basel, Switzerland), (−)-noradrenaline L-hydrogentartrate (Fluka Chemie AG, Buchs SG, Switzerland) and sodium nitroprusside (E. Merck AG, Darmstadt, Germany). Celiprolol was dissolved directly in tap water. The stock solutions of the compounds used in the *in vitro* studies were dissolved in distilled water. 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 were expressed as mean ± s.e.mean. The data were analysed with BMDP statistical software.

Results

Blood pressure, heart and body weights, and average medial thickness

In the beginning of the study the systolic blood pressures were already higher in SHR than in WKY rats, and during the 12-week-long follow up, blood pressures increased steadily in all SHR groups, whereas only a small elevation was observed in the two WKY groups. In those SHR that were treated with the higher dose of celiprolol (50 mg kg^{-1} day^{-1}) the rise in blood pressure was significantly attenuated, while the lower dose (5 mg kg^{-1} day^{-1}) was without a significant antihypertensive effect (Figure 1 and Table 1).

Celiprolol therapy did not significantly affect absolute heart or body weights in either strain. The heart/body weight ratio was clearly higher in untreated SHR than in WKY rats and it was unaffected by the lower dose of celiprolol. However, in SHR treated with the higher dose of celiprolol, the heart/body weight ratio did not significantly differ from that of untreated WKY rats (Table 1). Interestingly, treatment with either the low or the higher dose of celiprolol reduced the average medial thickness of the mesenteric artery in SHR, but no differences were observed between the two WKY rat groups (Figure 2 and Table 1).

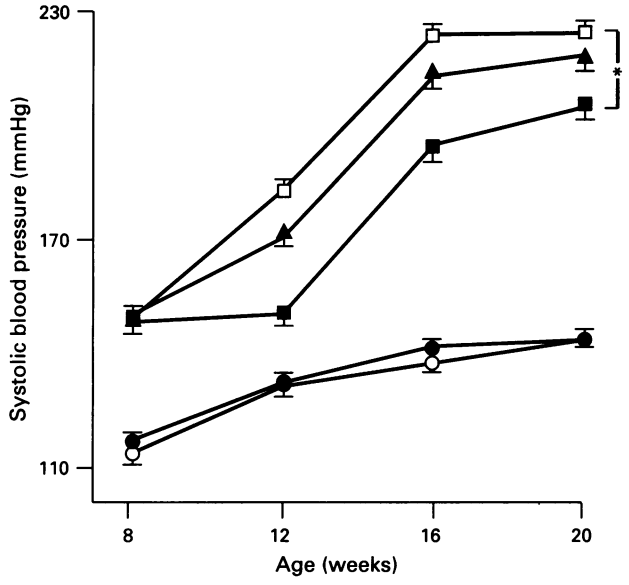

Figure 1 Blood pressure in untreated spontaneously hypertensive rats (SHR, □), celiprolol (5 mg kg^{-1} day^{-1})-treated SHR (▲), celiprolol (50 mg kg^{-1} day^{-1})-treated SHR (■), untreated Wistar-Kyoto (WKY, ○) rats, and celiprolol (50 mg kg^{-1} day^{-1})-treated WKY rats (●). Symbols indicate means with s.e.means, $n=10-12$ in each group; $*P<0.05$, ANOVA for repeated measurements.

Mesenteric arterial responses in vitro

The endothelium-mediated relaxations of NA-precontracted mesenteric arterial rings to ACh were markedly impaired in untreated SHR when compared with the two WKY groups. Interestingly, the response to ACh was clearly improved in SHR by treatment with either the low or the higher dose of celiprolol, but the relaxation still remained less marked than in WKY rats. Cyclo-oxygenase inhibition with diclofenac enhanced the relaxation to ACh in the three SHR groups ($P<0.02$ in all SHR groups), but did not affect the responses in the WKY rats. In contrast, the addition of the NO synthase inhibitor L-NAME to the organ bath practically abolished the relaxations to ACh in all SHR groups ($P<0.001$), whereas the responses in the WKY groups were slightly but significantly attenuated by L-NAME ($P<0.001$ in both WKY groups). Apamin, an inhibitor of Ca^{2+}-activated K^+ channels, had a moderate diminishing effect on the diclofenac and L-NAME-resistant relaxation to ACh in untreated WKY rats ($P<0.01$), while the response was not significantly affected in celiprolol-treated WKY rats (Figure 3).

When endothelium-mediated hyperpolarization of arterial smooth muscle was eliminated by precontraction induced by depolarization with 50 mM KCl, no differences were found between the study groups in the relaxation responses induced by ACh (Figure 4) or another endothelium-dependent agonist ADP (data not shown). In the presence of diclofenac the responses to ACh were somewhat augmented in the study groups ($P<0.04$) with the exception of celiprolol-treated WKY, while the addition of L-NAME almost completely abolished the relaxations to ACh in all study groups during KCl-induced precontractions ($P<0.001$; Figure 4).

The response to SNP, an agent that mediates arterial relaxation via the formation of exogenous nitric oxide and the subsequent accumulation of cyclic GMP, was attenuated in untreated SHR when compared with WKY rats. Therapy with either the low or the higher dose of celiprolol also improved this response in SHR, but the relaxation still remained less marked than that in the WKY rats (Figure 5). However, celiprolol treatment did not affect the vasorelaxation elicited by the β-adrenoceptor agonist, isoprenaline, in either strain (Figure 5). Arterial contractile sensitivity to NA and 5-HT in the absence and presence of diclofenac, and in the presence of diclofenac and L-NAME were comparable in all study groups (Table 2). The maximal contractile forces were also similar in the study groups (data not shown).

Table 1 Physiological variables in experimental groups at close of the study

	WKY	*Celi-WKY*	*SHR*	*LowCeli-SHR*	*Celi-SHR*
Systolic blood pressure (mmHg)					
beginning of treatment	114±3*	117±2*	150±3†	150±3†	149±3†
end of the study	144±2*	144±3*	225±3†	219±4†	206±4*†
Body weight (g)	407±7*	400±6*	369±8†	378±7†	370±9†
Heart weight (mg)	1222±20*	1104±20*	1411±78†	1377±41	1249±41
Heart/body weight (mg g^{-1})	2.99±0.04*	2.77±0.05*	3.84±0.22†	3.66±0.16†	3.37±0.05
Average medial thickness (μm)	71±2*	60±4*	91±2†	84±1*†	78±2*

Values are mean ± s.e.mean, $n=8-12$ for all groups. WKY and Celi-WKY, untreated and celiprolol (50 mg kg^{-1} day^{-1})-treated Wistar-Kyoto rats, respectively. SHR, LowCeli-SHR and Celi-SHR, untreated, celiprolol (5 mg kg^{-1} day^{-1})-treated and celiprolol (50 mg kg^{-1} day^{-1})-treated spontaneously hypertensive rats, respectively. $*P<0.05$ compared with SHR group, $†P<0.05$ compared with WKY group (Bonferroni test).

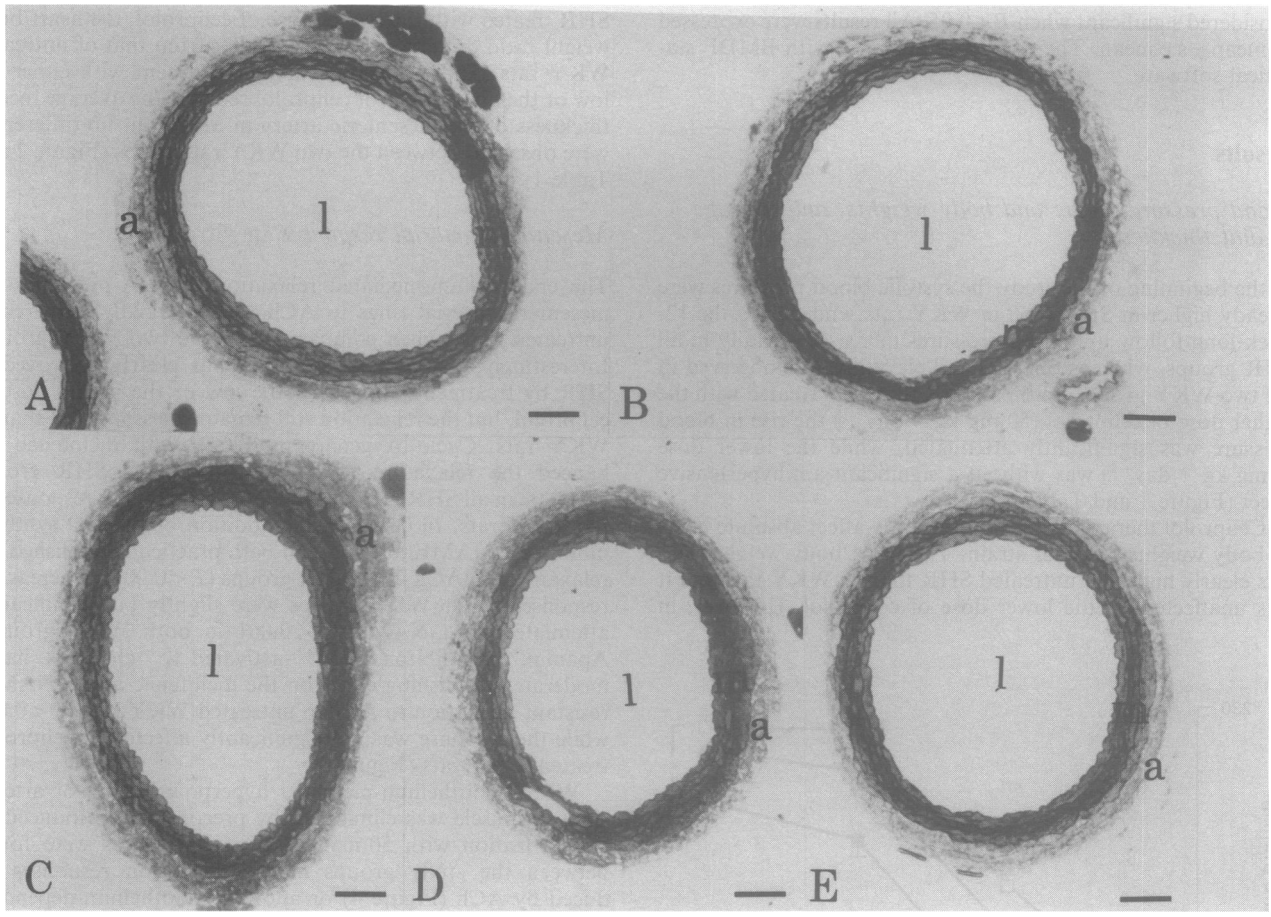

Figure 2 Light microscopy of representative mesenteric arterial rings from untreated Wistar-Kyoto (WKY, A) rats, celiprolol (50 mg kg^{-1} day^{-1})-treated WKY rats (B), spontaneously hypertensive rats (SHR, C), celiprolol (5 mg kg^{-1} day^{-1})-treated SHR (D) and celiprolol (50 mg kg^{-1} day^{-1})-treated SHR (E). a = adventitia, m = media, l = lumen; calibration bar = 100 μm.

Discussion

In the present study, therapy with the higher dose of celiprolol had a moderate antihypertensive action in SHR, whereas blood pressure was not significantly affected by the lower dose of the drug. However, heart weights were not decreased by celiprolol in SHR, suggesting that the reduction in the afterload that accompanied the moderate lowering of blood pressure was not sufficient to attenuate cardiac hypertrophy. In the normotensive WKY rats, celiprolol therapy had no effect on blood pressure. On the basis of previous reports, 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). Moreover, chronic β-adrenoceptor antagonism has been reported either to reduce moderately (Lauva & Tomanek, 1985; Ohlstein *et al.*, 1994) or not to affect heart weights in SHR (Chatelain *et al.*, 1981). Nevertheless, morphological studies of the vasculature revealed that media smooth muscle hypertrophy of the mesenteric artery was significantly reduced by both of the present doses of celiprolol in SHR. This suggests that the antihypertrophic property of celiprolol may not be solely related to the haemodynamic changes induced by the treatment, and that other factors such as inhibition of neurohumoral activation or cellular growth may also be involved. Indeed it has been suggested that the antihypertrophic effect of carvedilol on vascular smooth muscle is mediated by a combination of haemodynamic and anti-mitogenic effects (Ohlstein *et al.*, 1994).

ACh relaxes arteries in an endothelium-dependent manner via the release of the endothelium-derived relaxing factor (EDRF), which stimulates smooth muscle soluble guanylate cyclase and elevates intracellular cyclic GMP (Moncada *et al.*, 1991). EDRF is probably identical with nitric oxide (NO) (Moncada *et al.*, 1991). Previously, several studies have reported impaired endothelium-dependent relaxation in different forms of hypertension (Watt & Thurston, 1989; Treasure *et al.*, 1992; Kähönen *et al.*, 1994; Mäkynen *et al.*, 1996b), a perception which was also confirmed in the present study. We found that treatment of SHR with either low or higher dose of celiprolol was accompanied by a comparable enhancement of the endothelium-mediated relaxation response to ACh. The finding that diclofenac moderately improved the dilator response to ACh in the treated and untreated SHR but not in the WKY rats may indicate the predominance of vasoconstrictor prostanoids over the dilator prostacyclin in this type of hypertension. Similar improvement of the dilator response to ACh has been observed in SHR when using the cyclo-oxygenase inhibitor, indomethacin, as well as in deoxycorticosterone-NaCl induced hypertension when using diclofenac (Kähönen *et al.*, 1995b; Mäkynen *et al.*, 1996a). Interestingly, inhibition of NO-synthesis by L-NAME effectively diminished relaxations to ACh in all SHR and abolished the difference between the treated and untreated SHR, whereas both WKY groups still showed distinct L-NAME resistant relaxations. This suggests that the enhancement of endothelium-mediated relaxation achieved by treatment with both doses of celiprolol was largely mediated by endothelium-derived NO in SHR. Interestingly, celiprolol therapy has recently been reported to increase NO synthase activity in the neutrophils of patients with essential hypertension (Mehta *et al.*, 1994).

ACh has recently been shown to cause hyperpolarization of arterial smooth muscle *in vitro* (Chen *et al.*, 1988; Feletou & Vanhoutte, 1988; Bray & Quast, 1991; Garland & McPherson,

1992), while NO has also been proposed to activate Ca^{2+}-activated K^+ channels directly and thus cause hyperpolarization (Bolotina *et al.*, 1994). Several findings, however, suggest that the hyperpolarization induced by ACh is not mediated via NO (Fujii *et al.*, 1992; Garland & McPherson, 1992; Vanheel *et al.*, 1994), and the existence of a distinct endothelium-derived hyperpolarizing factor (EDHF) has been proposed although its exact identity still remains unknown (Chen *et al.*, 1988; Garland & McPherson, 1992; Fujii *et al.*, 1993; Li *et al.*, 1994). In the mesenteric artery of the rat, apamin has been found to reduce the L-NAME resistant endothelium-dependent relaxation by 55%, and to abolish completely the response when combined with another Ca^{2+}-activated K^+ channel blocker, charybdotoxin (Waldron & Garland, 1994). In contrast, the blocker of the ATP-sensitive K^+ channels, glyburide, was found to be ineffective (McPherson & Angus, 1991; Garland & McPherson, 1992). These findings indicate that EDHF causes relaxation of arterial smooth muscle via activation of Ca^{2+}-activated K^+ channels. In the present study, apamin somewhat attenuated the L-NAME- and diclofenac-resistant relaxation in untreated WKY rats, suggesting that this relaxation was at least partially mediated via Ca^{2+}-activated K^+ channels.

The membrane depolarization induced by precontracting the arterial preparations with KCl has been reported to eliminate the action of EDHF (Adeagbo & Triggle, 1993). Interestingly, no significant differences were found between SHR and WKY rats in response to ACh and ADP when the relaxations were elicited in KCl-precontracted rings. In the presence of diclofenac the relaxations to ACh and ADP were again augmented in all SHR groups. Moreover, the relaxations to ACh and ADP in KCl-precontracted preparations of all groups were practically abolished by L-NAME, suggesting that NO and EDHF were indeed responsible for the observed dilator responses to these agonists.

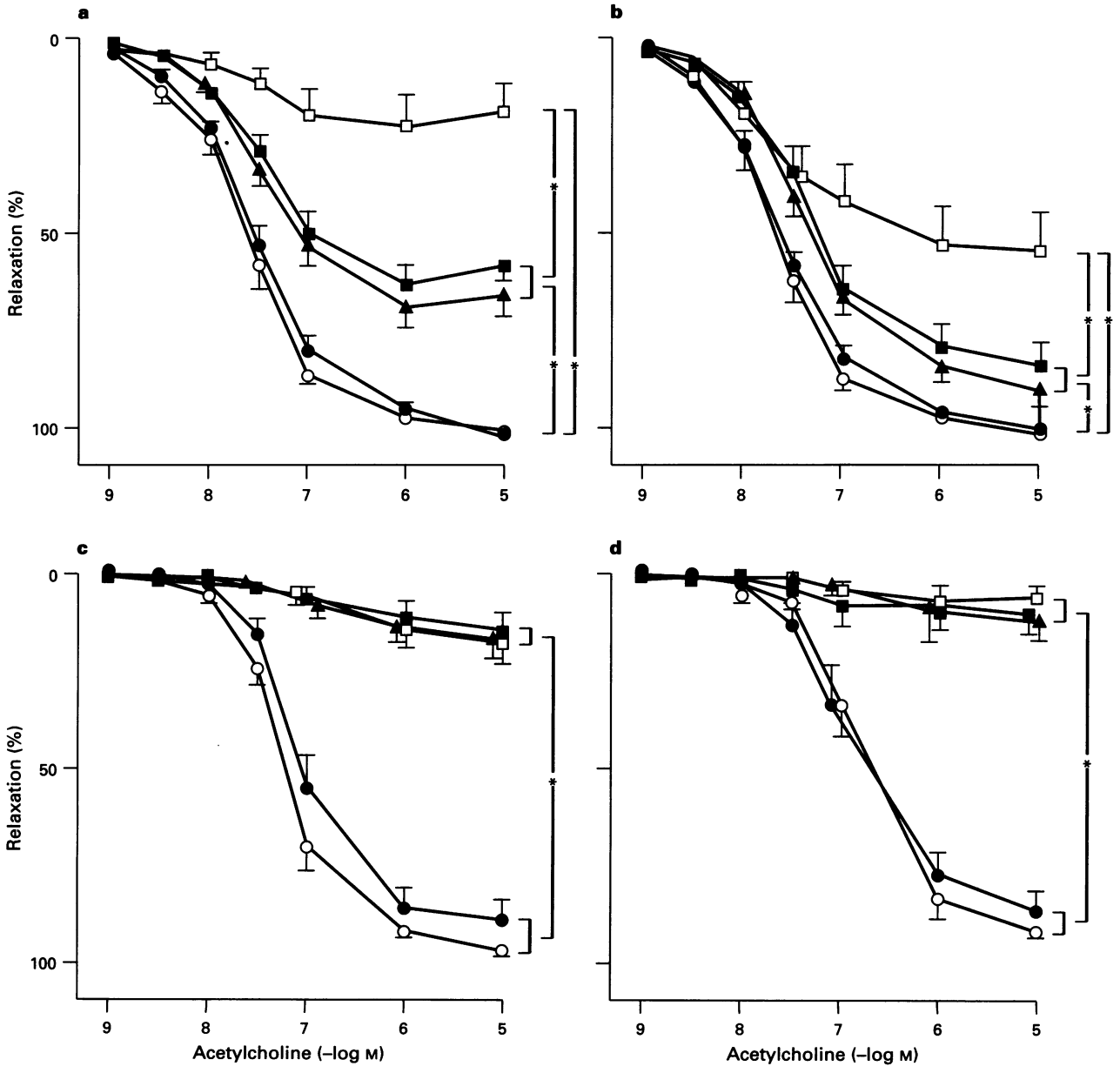

Figure 3 Relaxations to acetylcholine in isolated endothelium-intact mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR, □), celiprolol (5 mg kg^{-1} day^{-1})-treated SHR (▲), celiprolol (50 mg kg^{-1} day^{-1})-treated SHR (■), untreated Wistar-Kyoto (WKY, ○) rats, and celiprolol (50 mg kg^{-1} day^{-1})-treated WKY rats (●). The relaxations were induced after precontraction with 1 μM noradrenaline in the absence (a) and presence (b) of 3 μM diclofenac, in the presence of diclofenac and 0.1 mM N^G-nitro-L-arginine methyl ester (L-NAME, c), and in the presence of diclofenac, L-NAME and 1 μM apamin (d). Symbols indicate means with s.e.means, $n = 8-10$ in each group; $^*P < 0.05$, ANOVA for repeated measurements.

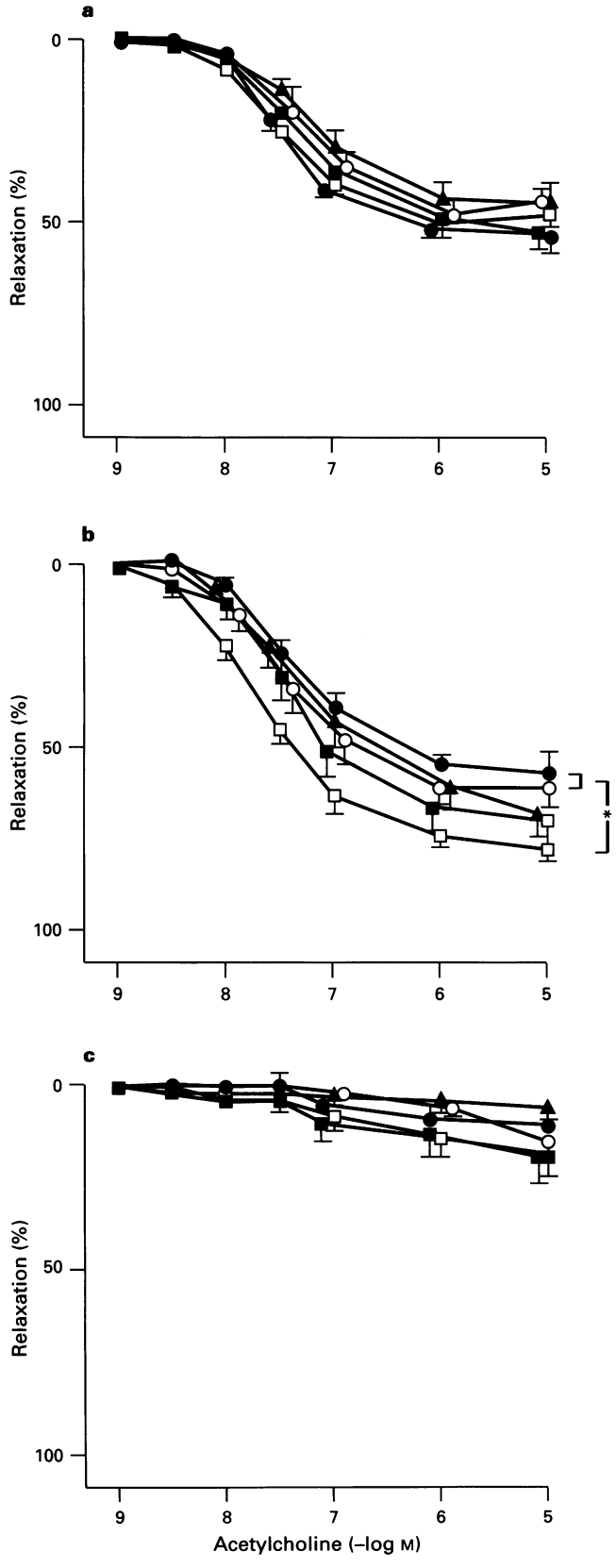

Figure 4 Relaxations to acetylcholine in isolated endothelium-intact mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR, □), celiprolol ($5\,mg\,kg^{-1}\,day^{-1}$)-treated SHR (▲), celiprolol ($50\,mg\,kg^{-1}\,day^{-1}$)-treated SHR (■), untreated Wistar-Kyoto (WKY, ○) rats, and celiprolol ($50\,mg\,kg^{-1}\,day^{-1}$)-treated WKY rats (●). The relaxations were induced after precontraction with 50 mM KCl in the absence (a) and presence (b) of 3 μM diclofenac, and in the presence of diclofenac and 0.1 mM N^G-nitro-L-arginine methyl ester (L-NAME, c). Symbols indicate means with s.e.means, $n=8-10$ in each group; $^*P<0.05$, ANOVA for repeated measurements.

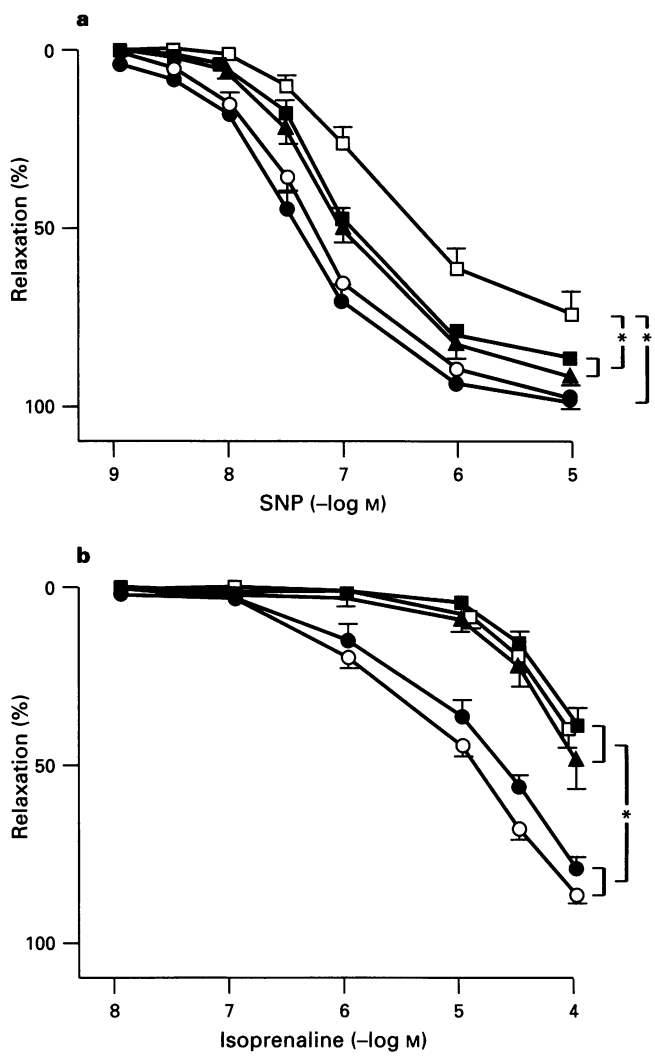

Figure 5 Relaxations to sodium nitroprusside (SNP, a) and isoprenaline (b) after precontraction with 1 μM noradrenaline in isolated endothelium-intact mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR, □), celiprolol ($5\,mg\,kg^{-1}\,day^{-1}$)-treated SHR (▲), celiprolol ($50\,mg\,kg^{-1}\,day^{-1}$)-treated SHR (■), untreated Wistar-Kyoto (WKY, ○) rats, and celiprolol ($50\,mg\,kg^{-1}\,day^{-1}$)-treated WKY rats (●). Symbols indicate means with s.e.means, $n=8-10$ in each group; $^*P<0.05$, ANOVA for repeated measurements.

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 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, has been suggested to augment the release of NO (Gray & Marshall, 1992; Graves & Poston, 1993). Yet, in the present study the vasorelaxation to isoprenaline remained comparable in the treated and untreated SHR, suggesting that celiprolol caused neither up- nor downregulation of β-adrenoceptors, nor did it affect the pathways leading from the activation of β-adrenoceptor to the production of cyclic AMP. In contrast, arterial relaxations elicited by SNP in SHR were similarly augmented by either dose of celiprolol, suggesting that the sensitivity of arterial smooth muscle to NO was enhanced by celiprolol treatment.

Previously, treatment with atenolol, as well as with angiotensin converting enzyme inhibitors have been shown to normalize the relaxation to ACh in SHR (Clozel *et al.*, 1990;

Table 2 Parameters of contractile responses of isolated mesenteric arterial rings

	WKY	*Celi-WKY*	*SHR*	*LowCeli-SHR*	*Celi-SHR*
5-Hydroxytryptamine					
pD_2					
	6.32±0.07	6.30±0.13	6.52±0.05	6.47±0.10	6.39±0.10
with diclofenac	6.13±0.07	6.19±0.11	6.16±0.08	6.28±0.09	6.30±0.10
with L-NAME and diclofenac	6.13±0.07	6.25±0.09	6.33±0.07	6.38±0.06	6.38±0.06
Noradrenaline					
pD_2					
	6.79±0.08	6.78±0.14	6.70±0.07	6.66±0.10	6.58±0.11
with diclofenac	6.59±0.07	6.65±0.10	6.33±0.10	6.46±0.08	6.48±0.09
with L-NAME and diclofenac	6.86±0.06	6.99±0.10	6.65±0.07	6.70±0.07	6.70±0.08

Values are mean±s.e.mean, $n=10-11$ in each group. WKY and Celi-WKY, untreated and celiprolol ($50\,mg\,kg^{-1}\,day^{-1}$)-treated Wistar-Kyoto rats, respectively. SHR, LowCeli-SHR and Celi-SHR, untreated, celiprolol ($5\,mg\,kg^{-1}\,day^{-1}$)-treated and celiprolol ($50\,mg\,kg^{-1}\,day^{-1}$)-treated spontaneously hypertensive rats, respectively. pD_2 is the negative logarithm of the concentration of agonist producing 50% of maximal contractile response. Significant differences were not found between the study groups.

Arvola *et al.*, 1993; Kähonen *et al.*, 1994), whereas diuretic therapy with trichlormethiazide only moderately augmented the response (Kähönen *et al.*, 1995a), while hydralazine treatment was without effect on 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 long-term antihypertensive treatment may not be related exclusively to the reduction of blood pressure, but to other mechanisms that affect endothelial and smooth muscle function as well. This view is further supported by the present study, since the improvement of ACh-induced relaxation was independent of the reduction of blood pressure.

Taken together, inhibition of NO synthase with L-NAME, and prevention of hyperpolarization by precontractions induced by KCl both eliminated the enhancement of the ACh-induced arterial relaxation in celiprolol-treated SHR. Since NO has been proposed to activate Ca^{2+}-activated K^+ channels directly (Bolotina *et al.*, 1994), these findings suggest that the improvement of endothelium-mediated relaxation observed was in fact mediated by enhanced hyperpolarization of arterial smooth muscle. This conclusion is further supported by the fact that the relaxations elicited by SNP were augmented by both doses of celiprolol in SHR, indicating that the sensitivity of arterial smooth muscle to NO was enhanced. Another possibility is that the above hyperpolarization mechanisms and increased NO production were both involved.

In conclusion, the present results suggest that therapy with celiprolol was accompanied by enhancement of endothelium-dependent and -independent NO-mediated arterial relaxation in SHR, and this effect appeared to be independent of the reduction of blood pressure. The augmented endothelium-mediated relaxation following celiprolol treatment may have been mediated by enhanced hyperpolarization of arterial smooth muscle, but increased endothelial production of nitric oxide may also have been involved.

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References

ADEAGBO, A.S.O. & TRIGGLE, C.R. (1993). Varying extracellular [K^+]: A functional approach to separating EDHF- and EDNO-related mechanisms in perfused rat mesenteric arterial bed. *J. Cardiovasc. Pharmacol.*, **21**, 423–429.

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.

BOLOTINA, V.M., NAJIBI, S., PALACINO, J.J., PAGANO, P.J. & COHEN, R.A. (1994). Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature*, **368**, 850–853.

BRAY, K. & QUAST, U. (1991). Differences in the K^+-channels opened by cromakalim, acetylcholine and substance P in rat aorta and porcine coronary artery. *Br. J. Pharmacol.*, **102**, 585–594.

BÜLBRING, E. & TOMITA, T. (1987). Catecholamine action on smooth muscle. *Pharmacol. Rev.*, **39**, 49–96.

CHATELAIN, P., WAELBROECK, M., CAMUS, J.-C., DE NEEF, P., ROBBERECHT, P., ROBA, J. & CHRISTOPHE, J. (1981). Comparative effects of α-methyldopa, propranolol and hydralazine therapy on caridac adenylate cyclase activity in normal and spontaneously hypertensive rats. *Eur. J. Pharmacol.*, **72**, 17–25.

CHEN, G., SUZUKI, H. & WESTON, A.H. (1988). Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. *Br. J. Pharmacol.*, **95**, 1165–1174.

CLOZEL, M., KUHN, H. & HEFTI, F. (1990). Effects of angiotensin converting enzyme inhibitors and of hydralazine on endothelial function in hypertensive rats. *Hypertension*, **16**, 532–540.

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.

FELOTOU, M. & VANHOUTTE, P.M. (1988). Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br. J. Pharmacol.*, **93**, 515–524.

FUJII, K., OHMORI, S., TOMINAGA, M., ABE, I., TAKATA, Y., OHYA, Y., KOBAYASHI, K. & FUJISHIMA, M. (1993). Age related changes in endothelium-dependent hyperpolarization in the rat mesenteric artery. *Am. J. Physiol.*, **265**, H509–H516.

FUJII, K., TOMINAGA, M., OHMORI, S., KOBAYASHI K., KOGA, T., TAKATA, Y. & FUJISHIMA, M. (1992). Decreased endothelium-dependent hyperpolarization to acetylcholine in smooth muscle of the mesenteric artery of spontaneously hypertensive rats. *Cir. Res.*, **70**, 660–669.

GARLAND, C.J. & MCPHERSON, G.A. (1992). Evidence that nitric oxide does not mediate the hyperpolarization and relaxation to acetylcholine in rat small mesenteric artery. *Br. J. Pharmacol.*, **105**, 429–435.

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.

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.

JANCZEWSKI, P., BOULANGER, C., IQBAL, A. & VANHOUTTE, P.M. (1987). Endothelium-dependent effects of carteolol. *J. Pharmacol. Exp. Ther.*, **247**, 590–595.

KÄHÖNEN, M., MÄKYNEN, H., ARVOLA, P. & PÖRSTI, I. (1994). Enhancement of arterial relaxation by long-term atenolol treatment in spontaneously hypertensive rats. *Br. J. Pharmacol.*, **112**, 925–933.

KÄHÖNEN, M., MÄKYNEN, H., ARVOLA, P., WUORELA, H. & PÖRSTI, I. (1995a). Arterial function after trichlormethiazide therapy in spontaneously hypertensive rats. *J. Pharmacol. Exp. Ther.*, **272**, 1223–1230.

KÄHÖNEN, M., MÄKYNEN, H., WU, X., ARVOLA, P. & PÖRSTI, I. (1995b). Endothelial function in spontaneously hypertensive rats: influence of quinapril treatment. *Br. J. Pharmacol.*, **115**, 859–867.

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.

LI, J., BIAN, K. & BUKOSKI, R.D. (1994). A non-cyclo-oxygense, non-nitric oxide relaxing factor is present in resistance arteries of normotensive but not spontaneously hypertensive rats. *Am. J. Med. Sci.*, **307**, 7–14.

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.

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.

MÄKYNEN, H., KÄHÖNEN, M., ARVOLA, P., WU, X., WUORELA, H. & PÖRSTI, I. (1996a). Endothelial function in deoxycorticosterone-NaCl hypertension: effect of calcium supplementation. *Circulation*, **93**, 1000–1008.

MÄKYNEN, H., KÄHÖNEN, M., WU, X., WUORELA, H. & PÖRSTI, I. (1996b). Reversal of hypertension and endothelial dysfunction in deoxycorticosterone-NaCl treated rats by high calcium diet. *Am. J. Physiol.*, **270**, (in press).

MAN IN'T VELD, A.J. (1991). Vasodilatation, not cardiodepression, underlies the antihypertensive effect of beta-adrenoceptor antagonists. *Am. J. Cardiol.*, **67**, 13B–17B.

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.

MCPHERSON, G.A. & ANGNUS, J. (1991). Evidence that acetylcholine mediated hyperpolarization of the rat small mesenteric artery does not involve the K^+ channel opened by cromakalim. *Br. J. Pharmacol.*, **103**, 1184–1190.

MEHTA, J.L., LOPEZ, L.M., CHEN, L. & COX, O.E. (1994). Alterations in nitric oxide synthase activity, superoxide anion generation, and platelet aggregation in systemic hypertension, and effects of celiprolol. *Am. J. Cardiol.*, **74**, 901–905.

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 adaptions during long-term β-adrenoceptor blockade; Studies with acebutolol, atenolol, pindolol, and propranolol in hypertensive patients. *Circulation*, **80**, 903–914.

MEIRACKER, A.H., MAN IN'T VELD, A.J., RITSEMA VAN ECK, H.J., BOOMSMA, F., SCHALEKAMP, M.A.D.H. (1988). Hemodynamic and hormonal adaptations to β-adrenoceptor blockade; A 24-hour study of acebutolol, atenolol, pindolol, and propranolol in hypertensive patients. *Circulation*, **78**, 957–968.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.

OHLSTEIN, E.H., VICKERY, L., ARLETH, A., BARONE, F., SUNG, C.P., CAMDEN, A., MCCARTNEYM L. (1994). Carvedilol, a novel cardiovascular agent, inhibits development of vascular and ventricular hypertrophy in spontaneously hypertensive rats. *Clin. Exp. Hypertens.*, **16**, 163–177.

STEINBERG, S.F., JAFFE, E.A. & BILEZIKIAN, J,P. (1984). Endothelial cells contain beta adrenoceptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **325**, 310–313.

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.

VANHEEL, B., VANDERVOORDE, J. & LEUSEN, I. (1994). Contribution of nitric oxide to the endothelium-dependent hyperpolarization in rat aorta. *J. Physiol.*, **475**, 277–284.

WALDRON, G.J. & GARLAND, C.J. (1994). Effect of potassium channel blockers on L-NAME insensitive relaxation in rat small mesenteric artery. *Can. J. Physiol. Pharmacol.*, **72** (Suppl. 1), 115.

WATT, P.A.C. & THURSTON, H. (1989). Endothelium-dependent relaxation in resistance vessels from the spontaneously hypertensive rats. *J. Hypertens.*, **7**, 661–666.

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