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Flow- and acetylcholine-induced dilatation in small arteries from rats with renovascular hypertension — effect of tempol treatment

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Abstract

We investigated whether renovascular hypertension alters vasodilatation mediated by nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) and the influence of the superoxide dismutase mimetic tempol on vasodilatation. One-kidney one-clip hypertensive Sprague–Dawley rats, treated with either vehicle or tempol (from weeks 5 to 10 after placement of the clip), and uninephrectomized control rats were investigated. In renal hypertensive rats systolic blood pressure increased to 171±6 mmHg (n=10), while in tempol-treated rats systolic blood pressure remained normal (139±7 mmHg, n=5). In isolated pressurized mesenteric small arteries NO-mediated dilatation was obtained by increasing flow rate and EDHF-mediated dilatation by acetylcholine. In arteries from hypertensive rats, flow-induced dilatation was blunted, as compared to normotensive and tempol-treated rats, while acetylcholine-induced dilatation remained normal. Measured by dihydroethidium staining there was an increased amount of superoxide in arteries from vehicle-treated rats, but not from tempol-treated rats. Expression by immunoblotting of endothelial NO synthase and the NAD(P)H oxidase subunit p47phox remained unaffected by high blood pressure and tempol treatment. Simultaneous measurements of NO-concentration and relaxation were performed in isolated coronary arteries from the same animals. As compared to vehicle-treated rats, both acetylcholine-induced relaxation and NO-concentration increased in arteries from tempol-treated animals, while only the relaxation was improved by the NO donor, S-nitroso-N-acetylpenicillamine (SNAP). In conclusion renovascular hypertension selectively inhibits flow-induced NO-mediated vasodilatation, while EDHF-type vasodilatation remains unaffected, suggesting that high blood pressure leads to increased generation of superoxide contributing to decreased NO bioavailability. Furthermore, the abnormal endothelium function can be corrected by tempol treatment, but this seems to involve mechanisms partly independent of NO.

Keywords: Nitric oxide; Endothelium; Renovascular hypertension; Tempol; NO microsensor

1. Introduction

Free oxygen radicals have attracted much attention as a contributing factor to the endothelial dysfunction associated with hypertension. One such free radical is superoxide (O_2^-) which can be generated by the NAD(P)H oxidase system in the vascular wall. Superoxide is degraded to hydrogen peroxide (H_2O_2) by superoxide dismutase, but may also react with nitric oxide (NO) resulting in the production of peroxynitrite ($ONOO^-$) thus decreasing the bioavailability of NO. A consequence of this would be a reduced NO-dependent vasodilatation (McIntyre et al., 1999).

A short transient pressure increase in isolated arteries is associated with increased superoxide production and diminished flow-mediated dilatation (Huang et al., 1998; Ungvari et al., 2003; Christensen et al., 2007). This abnormality can be corrected by inhibition of the NAD(P)H oxidase with apocynin (Hamilton et al., 2002; Ungvari et al., 2003) and the superoxide dismutase mimetic tempol (Christensen et al., 2007). Also long term high blood pressure *in vivo* is related to increased vascular superoxide production and alterations in endothelial function.

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This has been demonstrated in various models of high blood pressure such as the spontaneously hypertensive rat (Zalba et al., 2000) and the DOCA salt sensitive rat model of hypertension (Ghosh et al., 2004). Also rats with renovascular hypertension show impairment of NO-dependent vasodilatation due to superoxide production (Heitzer et al., 1999). Similar abnormalities have been described in patients with essential hypertension (Taddei et al., 1998) as well as renovascular mediated hypertension (Higashi et al., 2002). Also in rats with hypertension due to aortic banding the superoxide generating NAD(P)H oxidase is upregulated (Vaziri and Ni, 2005). Thus, the ubiquitously finding of an activated NAD(P)H system during hypertension suggests this to be due to high blood pressure *per se* rather than related to the cause of hypertension.

Previous studies have demonstrated that flow-mediated dilatation of mesenteric small arteries from normotensive rats is almost entirely dependent on NO, whereas receptor-mediated dilatation to acetylcholine possess the characteristics of endothelial derived hyperpolarizing factor (EDHF) type and relies on activation of Ca^{2+}-activated potassium channels (Edwards et al., 1998; Thorsgaard et al., 2003). This discrimination between flow- and acetylcholine-induced dilatation also seems to apply to human small arteries (Paniagua et al., 2001; Buus et al., 2000). The different mechanisms for flow- and acetylcholine-induced dilatation allowed us to investigate whether high blood pressure preferentially affects NO-dependent or EDHF-type vasodilatation. In contrast to the mesenteric small arteries, acetylcholine relaxation is NO-mediated in rat coronary small arteries (Symons et al., 2006), and intraluminal measurements of NO concentrations allow direct assessment of NO bioavailability (Simonsen et al., 1999).

Despite the well established role for superoxide as NO scavenger in endothelial dysfunction during high blood pressure conditions, it has not been studied whether receptor- and flow-mediated dilatation is affected in the same vascular preparation under similar experimental conditions. The primary aim of the present study was therefore to investigate to which extent high blood pressure in a rat model of renovascular hypertension influences NO-dependent flow-mediated dilatation or EDHF type acetylcholine-induced dilatation in isolated small arteries. Secondary, we tested whether scavenging of superoxide with tempol selectively affects either type of vasodilatation.

2. Methods

2.1. Animal model of hypertension

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). Male Sprague–Dawley rats (7–8 weeks, 180–200 g) were obtained from Møllegaard Breeding Center, Skensved, Denmark. The rats were anaesthesized with intraperitoneal midazolam (0.413 mg/kg), fentanyl (0.026 mg/kg) and fluanisone (0.825 mg/kg) and supplied with extra fluanisone if necessary. The right kidney was removed in all rats, and the left renal artery was either clipped (clip diameter 240 μm) or sham-operated as previously described (Stankevicius et al., 2002; Kvist and Mulvany, 2003). From weeks 5 to 10 after placement of the clip, renal hypertensive rats were treated with either vehicle ($n=10$) or tempol ($n=5$). Tempol dose was 1 mM in the drinking water which equalled approximately 86 mg/kg/day. Systolic tail blood pressure and heart rate was measured after 5 and 10 weeks using plethysmography (Stankevicius et al., 2002; Kvist and Mulvany, 2003). Rats were excluded if the clip around the left renal artery could not be identified after sacrificing the animals ($n=3$), leaving 10 rats in the normotensive control group, 7 rats in the vehicle-treated group and 5 rats in the tempol-treated group.

2.2. Perfusion myography

Mesenteric small arteries with diameters around 300 μm were dissected and cannulated on two micropipettes in the flow direction in a microvascular myograph (Danish Myotechnology, Aarhus, Denmark), equipped for measurements of pressure and generation of flow as previously described in detail (Thorsgaard et al., 2003; Christensen et al., 2007). The middle part of the vessel segment was viewed through an inverted microscope and the internal diameter was continuously recorded by a video camera. The signal was fed to a dimension-analyzing program (VesselView, Danish Myotechnology, Aarhus, Denmark) for subsequent analysis. The hydrostatic pressures of both inlet and outlet reservoirs were measured by transducers connected to the perfusion lines and the height of the reservoirs was regulated such that the intraluminal pressure was kept at 50 mmHg.

Two different levels of flow, 25–50 μl/min and 100–200 μl/min, were generated by regulating suction trough the vessel with a peristaltic pump. This resulted in shear stress levels of approximately 8 and 24 dyn/cm^2 as calculated from the following equation $\tau=4\eta Q/\pi r^3$, where τ is shear stress, η is fluid viscosity, Q is the average flow rate and r is the internal vessel diameter. The suction from the peristaltic pump created a pressure variation with a frequency of 0.04–0.3 Hz.

All experiments were performed in the presence of 3 μM indomethacin. The vessel was contracted to 60% of the resting diameter by adding 9,11-dideoxy-11α, 9α-epoxymethano prostaglandin $F_{2\alpha}$ (U46619, 10–40 nM) to both perfusion and chamber solutions and flow-mediated vasodilatation was measured at two different flow levels as described above. Each flow response was obtained for exactly 300 s and in between the two flow responses the vessel was allowed to regain the preconstriction level. Following the flow responses the vessel diameter was allowed to stabilize and then dilatation to increasing concentrations of acetylcholine (300 s per concentration) was recorded. Then the bath solution was changed 9–10 times with physiological saline solution and the responses to flow and acetylcholine were repeated as explained above.

2.3. Measurement of superoxide production

For assessment of superoxide production, mesenteric small arteries were quick-frozen in liquid nitrogen, sections were

obtained in a freeze microtome, and stained with dihydroethidium as previously described (Christensen et al., 2007). Fluorescence was evaluated with a confocal microscope (ODYSSEY XL, Noran, Japan) equipped with a water immersion objective (×60, numerical aperture 1.2, Nikon, Japan). Fluorescence images were quantified by use of MATLAB™ extended with the image processing toolbox (www.mathworks.com). Laser settings were identical for each image obtained. Fluorescence from elastic membranes was subtracted and fluorescence persisting in control preparations treated with polyethylene glycol superoxide dismutase (PEG-SOD) was used to subtract background fluorescence.

2.4. Immunoblotting

From each animal, two arterial segments of 3 mm length were quick-frozen in liquid nitrogen and kept at −80 °C until protein expression analysis and western blots for eNOS and p47 NAD(P)H oxidase subunit were performed. Protein concentrations were measured using a non-interfering protein assay kit (Calbiochem, CA, USA). Samples, containing an equal amount of protein, were loaded on 4–12% polyacrylamide Bis-Tris criterion gels (Bio-Rad Laboratories, Hercules, CA, USA) and protein separation was carried out in the Criterion Electrophoresis System (Bio-Rad Laboratories, Hercules, CA, USA) using XT MOPS as running buffer. Proteins were blotted onto a polyvinylidine difluoride (PVDF) membrane for 1 h at 100 mV and the membranes were incubated as follows: first they were blocked for 1 h in 5% milk, then the primary antibody was added in the right dilution (in 5% milk) and left rotating at 4 °C overnight. The next day the membranes were washed three times 10 min in tris buffered saline tween (TBS-T) or phosphate buffered saline tween (PBS-T) before the secondary antibodies were added (anti-mouse or -rabbit also in 5% milk). After incubating for 1 h at room temperature the membranes were again washed in TBS-T or PBS-T and then developed using an enhanced chemiluminescence detection system; ECL or ECL+ (Amersham Biosciences, England). The immunocomplexes were detected using an enhanced chemiluminiscence system (ECL Plus, Amersham International plc, Little Chalfont, UK) and scanned with the "Molecular Dynamics Storm" (Molecular Dynamics, Sunnyvale, CA). Signals on the immunoblot were quantified using ImageQuant® Software (GE Healthcare, Copenhagen, Denmark). The primary antibodies used were anti-human endothelial NO synthase (eNOS, Abcam, Cambridge, UK), anti-p47phox (1:200, Santa Cruz Biotechnology) and anti-human β-actin clone AC-15 (Abcam, Cambridge, UK). Data for protein expression are given as a ratio of α-actin expression, which was determined on the same membrane by using a monoclonal antibody (1:300.000, Boehringer Mannheim, Germany).

2.5. Simultaneous measurements of NO production and vasorelaxation

To evaluate the effect of renovascular hypertension and tempol treatment on small artery NO production we utilized isolated segments of the left proximal coronary artery (diameter 400–450 μm). As mentioned acetylcholine relaxation in mesenteric small arteries is mainly NO independent. However, relaxation to acetylcholine in similar sized coronary arteries is completely NO dependent (Symons et al., 2006). We therefore performed simultaneous measurements of force and NO concentration in coronary arteries mounted in a wire myograph as previously described (Simonsen et al., 1999). An NO sensitive microelectrode (ISONOP30, World Precision Instruments, Stevenage, UK) was introduced into the lumen of the coronary small arteries and recordings were obtained during relaxation with acetylcholine and the NO donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP) in U46619-contracted preparations. The effect of the NOS inhibitor asymmetric dimethyl L-arginine (ADMA, 300 μM) was tested on acetylcholine-mediated relaxation. Calibration with an NO gas solution and selectivity testing of the electrodes was performed as previously described (Simonsen et al., 1999).

2.6. Solutions and drugs

The arteries were held in a physiological salt solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $CaCl_2$ 2.5, EDTA 0.026, and glucose 5.5. PSS was kept at 37 °C, pH 7.4, and bubbled with 5% CO_2 in O_2. 9,11-dideoxy-11α, 9α-epoxymethano prostaglandin $F_{2\alpha}$ (U46619), acetylcholine-HCl, 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (tempol), S-nitroso-N-acetyl-D, L-penicillamine (SNAP) were obtained from Sigma (MO, USA).

2.7. Calculations and statistical evaluation

Responses are expressed as means ± S.E.M., where *n* equals the number of rats. Vasodilatation in the pressure myograph is expressed as area under the relaxation curve (AUC) for each response (300 s) to flow, acetylcholine or SNAP. Vasorelaxation in the wire myograph is expressed as percentage of the preconstriction level. Differences between means were compared by two-way analysis of variance (ANOVA). A value of $P<0.05$ was considered statistical significant.

3. Results

3.1. Blood pressure measurements

Five and 10 weeks after placement of the clip systolic blood pressure was significantly elevated in one-kidney, one-clip (1K1C) rats compared to sham operated normotensive control rats (Fig. 1A). Tempol treatment from weeks 5 to 10 after placement of the clip lowered systolic blood pressure to the level of the normotensive group, while blood pressure in the group receiving vehicle remained elevated (Fig. 1A). There were no differences in heart rate among the three experimental groups (Fig. 1B) and no significant differences in body weight, heart weight or kidney weight between the three groups (data not shown).

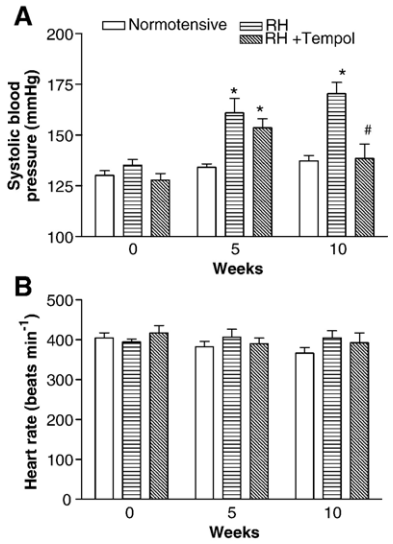

Fig. 1. Systolic blood pressure (A) and heart rate (B) in sham-operated normotensive rats ($n=10$) and renal hypertensive rats treated either with vehicle (RH, $n=7$) or tempol ($n=5$) from weeks 5 to 10 after placement of the clip. *$P<0.05$ vs. normotensive, $^{\#}P<0.05$ vs. vehicle treatment.

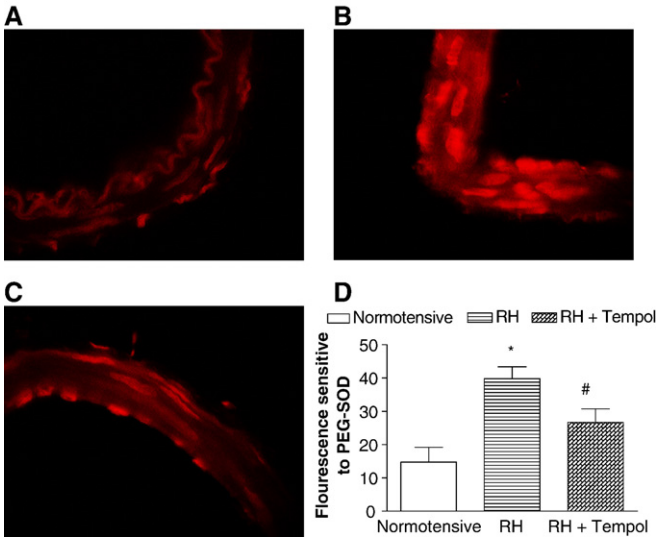

Fig. 3. Representative fluorescent photomicrographs of sections of rat mesenteric arteries from a normotensive rat (A), a renal hypertensive rat treated with vehicle (B), and a renal hypertensive rat treated with tempol (C). Quantification of fluorescence sensitive to superoxide dismutase (SOD) in the three groups of rats (D). *$P<0.05$ vs. normotensive, $^{\#}P<0.05$ vs. vehicle treatment, $n=5$–7 in each group.

3.2. Effect of renal hypertension on vasodilatation in mesenteric small arteries

Vasodilatation to both high and low shear stress was lower ($P<0.05$, $n=7$–10) in renal hypertensive rats as compared to sham-operated control rats (Fig. 2A), but conserved in arteries from rats treated with tempol ($n=5$, Fig. 2A). Vasodilatation induced by acetylcholine was normal in vehicle-treated renal hypertensive rats, and also remained unchanged in arteries from tempol-treated renal hypertensive rats (Fig. 2B). There were no differences in active (during U46619 constriction) or passive pressure–diameter relationships between renal hypertensive and normotensive rats (data not shown).

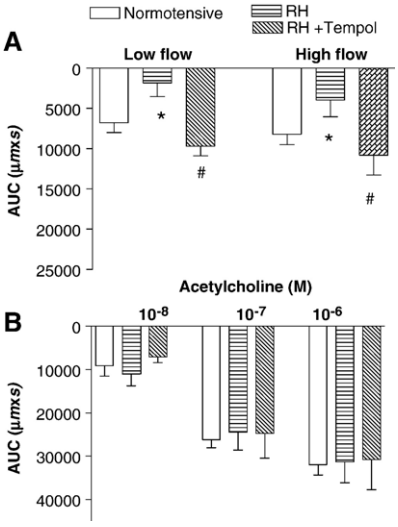

Fig. 2. Vasodilatation to flow (A) and acetylcholine (B) in arteries from normotensive and renal hypertensive rats (RH) treated with vehicle or tempol. *$P<0.05$ vs. normotensive, $^{\#}P<0.05$ vs. vehicle treatment, $n=5$–10 in each group.

3.3. Effect of renal hypertension on superoxide in mesenteric small arteries

The dihydroethidium fluorescence signal was increased in artery segments from renal hypertensive as compared to normotensive control rats and tempol-treated renal hypertensive rats (Fig. 3A–D). Incubation with PEG-SOD lowered the fluorescence level in arterial segments from all three groups of

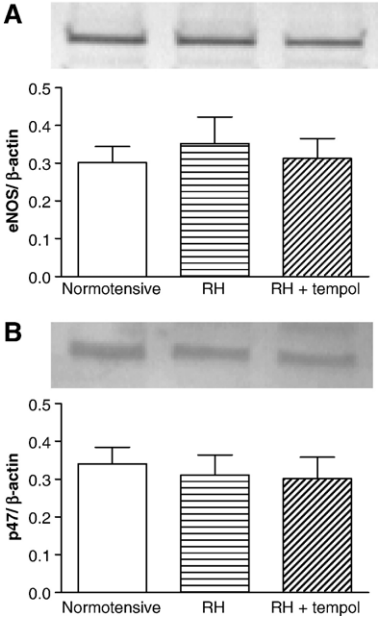

Fig. 4. Expression of eNOS (A) and the NAD(P)H oxidase subunit p47phox (B) in normotensive, vehicle- and tempol-treated renal hypertensive rats (RH). $n=5$–10 in each group.

rats confirming that dihydroethidium fluorescence reflects superoxide formation (Fig. 3D).

3.4. Effect of renal hypertension on expression of eNOS and p47phox in mesenteric small arteries

Immunohistochemistry revealed localization of NOS in the endothelial cell layer only, and that NOS was not detectable in control preparations where the primary antibody was omitted. Quantification of eNOS expression by Western blot showed no differences between artery segments from normotensive, vehicle and tempol-treated renal hypertensive rats (Fig. 4A). Immunohistochemistry for the NAD(P)H oxidase subunit p47phox showed localization primarily in the endothelial and adventitial layers, while there was only sparse immunoreactivity in the smooth muscle layer (results not shown). Quantification by Western blot revealed similar expression of p47phox in arterial segments from normotensive, vehicle and tempol-treated renal hypertensive rats (Fig. 4B).

3.5. Effect of renal hypertension on NO concentration in coronary arteries

In U46619-constricted arteries, acetylcholine induced concentration-dependent relaxation with a simultaneous increase in the NO concentration (Fig. 5A and B). In arteries from renal hypertensive rats, the acetylcholine-induced relaxation and increase in NO concentration were markedly reduced ($P<0.05$, $n=5–6$, Fig. 5A and B). Tempol treatment normalized the acetylcholine-induced relaxation (Fig. 5B), although the increase in NO concentration still remained reduced (Fig. 5A). Relaxation to the NO donor SNAP was also reduced in the renal hypertensive rats as compared to normotensive and tempol-treated hypertensive rats (Fig. 5D). The reduction in relaxation was seen despite of equal concentrations of NO within the vascular lumen (Fig. 5C).

The relaxation to acetylcholine was completely abolished by ADMA (300 μM) in renal hypertensive rats and normotensive

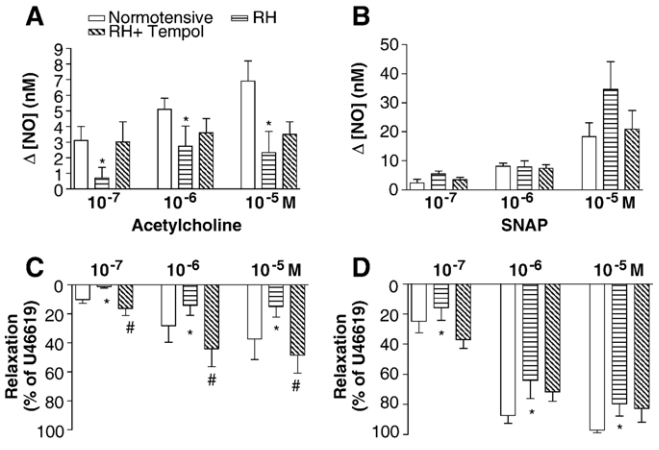

Fig. 5. Simultaneous measurements of changes in [NO] (A+B) and relaxation (C+D) induced by acetylcholine and SNAP in coronary arteries from normotensive rats and renal hypertensive rats (RH) treated with either vehicle or tempol. *$P<0.05$ vs. normotensive, #$P<0.05$ vs. vehicle treatment, $n=5–10$ in each group.

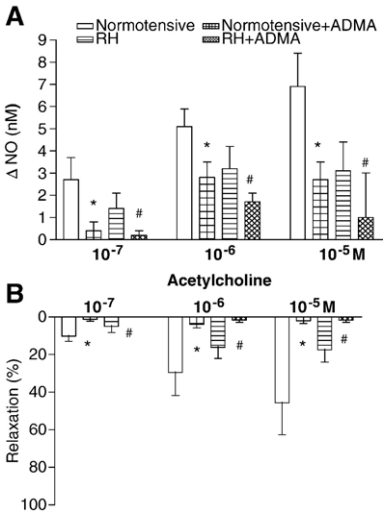

Fig. 6. Effect of asymmetric dimethyl L-arginine (ADMA, 300 μM) on simultaneous measurements of changes in [NO] (A) and relaxation (B) induced by acetylcholine in coronary arteries from normotensive and renal hypertensive (RH) rats. *$P<0.05$ vs. no ADMA, #$P<0.05$ vs. no ADMA, $n=5–10$ in each group.

rats with a concomitant reduction in the increase in NO concentration (Fig. 6A–B).

4. Discussion

The present study is the first to directly compare NO- and non-NO-dependent dilatation in isolated arteries from hypertensive animals under similar experimental conditions. We confirm the finding from several previous studies that high blood pressure results in impaired flow-mediated vasodilatation (Huang et al., 1998; Ungvari et al., 2003), but also demonstrates that the EDHF type dilatation remains normal. Thus, the main findings of the present study are as follows: (1) Renovascular hypertension in rats selectively impairs NO-mediated vasodilatation in small arteries probably through increased formation of superoxide, while EDHF type vasodilatation remains unaffected. (2) Treatment with the superoxide dismutase mimetic, tempol, lowers blood pressure and formation of superoxide. (3) Measurements with the NO microsensor demonstrated that endothelial NO release is reduced in arteries from renal hypertensive animals, but that tempol restores vasodilatation without a concomitant increase in the NO concentration. These findings together with unaltered expression of eNOS and p47 subunit of NAD(P)H oxidase suggest that high blood pressure *per se* increases superoxide formation leading to decreased NO availability. This results in impairment of flow-mediated dilatation in mesenteric arteries and acetylcholine-induced relaxation in coronary arteries.

We found that oral tempol treatment effectively reduced blood pressure to the level of the normotensive control rats. This is in accordance with findings from a similar rat model of renovascular hypertension (Dobrian et al., 2001). The blood pressure lowering effect of tempol may partly be ascribed to an increased peripheral vasodilatatory capacity due to improved NO availability (Schnackenberg et al., 1998). However, also

lowering of central sympathetic nervous activity can contribute to blood pressure reduction independently of changes in superoxide production (Xu et al., 2004).

It is well established that flow-mediated dilatation of mesenteric small arteries is dependent on endothelial-derived NO and blockade of NOS effectively attenuates the response (Thorsgaard et al., 2003). As opposed to receptor-mediated stimulation, shear stress only elicits a transient increase in endothelial cell calcium concentration. Instead eNOS can be activated by partially calcium-independent mechanisms through a tyrosine kinase sensitive to herbimycin A (Fleming et al., 1998; Thorsgaard et al., 2003). To which extent this pathway is affected by high pressure is unknown and whether the production of NO within the endothelial cell is altered in the hypertensive rats can be difficult to establish. However, measurements of eNOS expression in the mesenteric arteries did not suggest this to be the case (Fig. 4). Furthermore, we have previously found normal vasodilatation to guanylyl cyclase activation in arteries exposed to a transient pressure increase (Christensen et al., 2007) suggesting that the effect of NO on the smooth cells remains unaffected by high pressure. This is also supported in the present study by the results obtained with SNAP in coronary arteries from tempol-treated rats (Fig. 5). In contrast to the small arteries investigated in the present study, Barton et al. (2001) found significant upregulation of eNOS in aorta in the aortic coarctation model of hypertension suggesting a different effect of high blood pressure in a large vessel. Our results demonstrate that the main effect of high pressure seems to be scavenging of NO by free radicals rather than impairment of the NO signalling pathway.

So far it has been technical impossible to perform simultaneous measurements of NO concentrations and vessel diameter during flow-mediated dilatation. We therefore applied the NO microelectrode technique to wire-mounted coronary arteries. In these vessels, as opposed to the mesenteric arteries, acetylcholine-mediated relaxation is completely NO-dependent (Symons et al., 2006). Our study is the first to directly suggest that hypertension reduces acetylcholine-induced NO release to the vascular lumen. The reduction in SNAP-induced relaxation in hypertensive rats despite sufficient intraluminal concentrations of NO further demonstrates scavenging of NO in the vascular wall.

An important question is how high blood pressure increases the superoxide content of the vascular wall. The amount of NAD(P)H oxidase, measured here as the p47phox subunit, does not seem to be upregulated. However, this in contrast to findings from aorta of spontaneously hypertensive rats (Lodi et al., 2006) and the aortic coartation model of hypertension (Vaziri and Ni, 2005) suggesting that large and small vessels may be affected differently by high blood pressure. Instead our results may be explained by an enhanced activity of the enzyme or a reduced dismutation of superoxide. Previous studies have shown that a short term pressure elevation for one hour in pressurized small mesenteric arteries from normotensive rats results in impairment of flow-mediated NO-dependent dilatation to the same degree as demonstrated in the renal hypertensive rats (Christensen et al., 2007). In these short term pressurized vessels inhibition of the NAD(P)H oxidase with apocynin resulted in normalization of flow-mediated dilatation and furthermore this effect could be abolished by an inhibitor of NO synthase (unpublished results). These findings may point to a rapid increase in the activity of the NAD(P)H oxidase elicited by high intravascular pressure. This is supported by the observation that tempol normalizes flow-mediated dilatation in short term pressurized mesenteric vessels (Christensen et al., 2007) as well as in arteries from renal hypertensive rats.

Numerous studies have compared acetylcholine-induced vasodilatation in rat or human small pressurized arteries from hypertensive and normotensive individuals, but the results are not uniform. Some find a small although significant attenuation of the acetylcholine response (Falloon and Heagerty, 1994; Park et al., 2002), whereas others detect no differences under similar experimental conditions (Dohi et al., 1991), as was also the case in the present study. The discrepancies may be due to the model of hypertension used or the mode of vessel preconstriction. Furthermore, with decreasing vessel size non-NO-mediated dilatation contributes more to the acetylcholine response as shown for the rat mesenteric vascular bed by Tomioka et al. (1999).

Both in the mesenteric as well as the coronary arteries tempol tended to improve endothelial function of hypertensive rats beyond that observed in the normotensive rats, although tempol did not scavenge superoxide to the level of the normotensive rats (Fig. 3). However, beside the conversion of superoxide to hydrogen peroxide, tempol may also possess other properties including scavenging of vasocontracting hydroxyl radicals (˙OH) (Li et al., 2004) and direct vasodilatating properties through activation of BK channels (Xu et al., 2005). Furthermore, tempol normalized acetylcholine-induced relaxation in coronary arteries without appearing to restore NO levels, indicating that tempol can improve endothelium function through mechanisms independent of NO. A possible limitation to this finding is that the NO-microsensor technique used only measures changes in the NO concentration and not the absolute level. However, the baseline NO concentration in arteries from renal hypertensive rats is reduced (Stankevicius et al., 2002) and it is unlikely that tempol should increase NO levels above what is seen in the normotensive rats. Thus, taken together tempol improves endothelial function in renal hypertensive rats at least partly through mechanisms not directly related to restoring NO-levels.

In conclusion the present study demonstrates that renovascular hypertension selectively inhibits flow-induced NO-mediated vasodilatation, while EDHF-type vasodilatation remains unaffected, suggesting that high blood pressure leads to increased generation of superoxide contributing to decreased NO bioavailability. Furthermore, the abnormal endothelium function can be corrected by tempol treatment, but this seems to involve mechanisms partly independent of NO.

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