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Decreased basal despite enhanced agonist-stimulated effects of nitric oxide in 12-week-old stroke-prone spontaneously hypertensive rat

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Abstract

This study examined both basal and agonist-stimulated effects of nitric oxide in rings of thoracic aorta and carotid artery from 12-week-old stroke-prone spontaneously hypertensive rats (SHRSP) and compared them to those found in rings from normotensive Wistar Kyoto (WKY) controls. Acetylcholine-induced endothelium-dependent relaxation was found to be five-fold more sensitive in both male and female SHRSP when compared with those from age- and sex-matched WKY rats. In contrast, we found a reduction in the effects of basal nitric oxide in the SHRSP rat. Specifically, the ability of basal nitric oxide to depress contractile responses to phenylephrine was found to be reduced in vessels from SHRSP when compared with those from WKY rats. In addition, the endothelium-dependent depression of vasodilator responses to the nitric oxide donor, glyceryl trinitrate, was reduced in vessels from SHRSP when compared to those from WKY rats. Thus, we have shown that the effects of basal nitric oxide are impaired in the SHRSP rat at an age when the effects of agonist-stimulated nitric oxide are actually enhanced. This impairment may be related to the greater susceptibility of basal nitric oxide to destruction by superoxide anion which is known to be produced in excess in this model of hypertension.

Keywords: Stroke-prone spontaneously hypertensive rats (SHRSP); Nitric oxide (NO); Endothelium

1. Introduction

Nitric oxide is released from the vascular endothelium basally, i.e., in the absence of stimulation and exerts a vasodilator action in the vasculature (for review see Moncada et al., 1991a). This tonic dilator action often results in contractile responses to vasoconstrictors being depressed in endothelium-containing vessels when compared to those in which the endothelium has been removed. The same phenomenon is responsible for the enhancement of tone in endothelium-containing but not endothelium-denuded vessels following treatment with agents such as haemoglobin, the scavenger of nitric oxide, methylene blue, an inhibitor of soluble guanylate cyclase and N^G-nitro L-arginine methyl ester (L-NAME), and N^G-monomethyl L-arginine (L-NMMA), inhibitors of nitric oxide synthase (Konishi and Su, 1983; Egleme et al., 1984; Martin et al., 1986; Rees et al., 1990). In addition to its ability to depress vasoconstriction, it has been clearly demonstrated in endothelium-containing tissues that basal nitric oxide activity also opposes vasodilator responses induced by stimulants of soluble guanylate cyclase (Shirasaki and Su, 1985) including sodium nitroprusside (Moncada et al., 1991b), peroxynitrite (Dowell and Martin, 1998) and 3-morpholono-sydnonimine (SIN-1) (Busse et al., 1989; Flavahan and Vanhoutte, 1989; Luscher et al., 1989). This depression too is abolished by endothelial removal or blockade of the effects of nitric oxide.

It is thought that the effects of basal nitric oxide are vital in maintaining vascular tone and is perhaps more important than agonist-stimulated effects in controlling resting blood pressure (Moncada et al., 1991a). There is good evidence to suggest that the effects of basal nitric oxide are reduced in human essential hypertension (Linder et al., 1990; Panza et al., 1990; Taddei et al., 1993) and in some animal models of hypertension (Osugi et al., 1990; Maruyama and Maruyama, 1994; Rees et al., 1996), with the resultant loss of vasodilation, resulting in an increase in vascular tone and, consequently, in blood pressure. In the

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stroke-prone spontaneously hypertensive rat (SHRSP), an animal model of hypertension, there have been several reports demonstrating alterations in the effects of both agonist-stimulated and basal nitric oxide. For example, it has been demonstrated that in young animals (8 weeks) agonist-stimulated nitric oxide is enhanced (Onda et al., 1994; Tomita et al., 1995), whereas in older animals (16 weeks plus) both agonist-stimulated and basal nitric oxide effects are reduced (Osugi et al., 1990; Matsuda et al., 1995; Kitazono et al., 1996). In previous studies from our own laboratory we have reported that nitric oxide synthase activity and mRNA is enhanced at 16 weeks of age despite a functional reduction in agonist-stimulated nitric oxide-dependent relaxation (McIntyre et al., 1997; Kerr et al., 1999). In addition, we demonstrated a reduced ability of L-NAME, a nitric oxide synthase inhibitor, to elevate tone induced by a single concentration of phenylephrine (EC_{20}) in thoracic aorta from SHRSP when compared with those from normotensive control Wistar Kyoto rats (WKY) (McIntyre et al., 1997), suggesting a deficiency of basal nitric oxide in the hypertensive strain. This effect was most marked in males.

The aim of this study, therefore, was to examine more thoroughly the possibility that the effects of basal nitric oxide are reduced in the SHRSP rat when compared to WKY controls. In order to test this, the endothelium-dependent depression of vasoconstriction by basal nitric oxide was assessed by constructing full concentration–response curves to the contractile agonist phenylephrine in the presence and absence of the endothelium and in the presence and absence of the nitric oxide synthase inhibitor, L-NAME. In addition the endothelium-dependent depression of vasodilation by basal nitric oxide was assessed by constructing full concentration–response curves to the nitric oxide-donor glyceryl trinitrate. In each case, two vessels, i.e., thoracic aorta and carotid artery, were examined to determine if any differences seen in the two strains were localised to a particular artery.

2. Materials and methods

2.1. Experimental animals

WKY and SHRSP were obtained from colonies established in Glasgow by brother–sister mating as previously described (Dominiczak et al., 1993). The breeding stocks were obtained from colonies maintained at the University of Michigan which had in turn obtained its breeding stocks from the National Institute of Health. All rats were kept in the same environment and received food and water ad libitum. At 11 weeks of age, indirect blood pressure was measured by tail plethysmography in conscious, restrained animals habituated to the procedure as previously described (Evans et al., 1994). Rats were pre-warmed to 34°C for 10 to 15 min before measurements. Rats were studied at 12 weeks of age and were killed by an intraperitoneal injection of Euthetal (150 mg kg^{-1}).

2.2. Preparation of vessel rings and tension recording

The preparation of rings of thoracic aorta and carotid artery for tension recording was essentially similar to that described by Martin et al. (1985). The vessels were removed, cleared of adhering fat and connective tissue and cut into 2.5 mm wide transverse rings with a razor blade slicing device. Endothelial cells were removed from some rings by gently rubbing the intimal surface (30–60 s) with a moist stick. Successful removal of the endothelium was later confirmed by the inability of acetylcholine (1 μM) to elicit relaxation. The vessel rings were mounted under 1 g resting tension on stainless steel hooks in 10-ml organ baths, and bathed at 37°C in Krebs solution containing (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24, D-glucose 11 and gassed with 95% O_2 and 5% CO_2. Tension was recorded isometrically with Grass FT03 transducers and displayed on a PowerLab (800 Series, AD Instruments). Tissues were allowed to equilibrate for 60 min before experiments were begun, during which time the resting tension was re-adjusted to 1 g if required.

2.3. Experimental protocols

After equilibration, each preparation was stimulated with 100 mM KCl. This contractile response was taken as an index of arterial contractility and subsequent contractile responses were expressed as a percentage of this KCl-evoked contraction. Following washout and re-equilibration, a cumulative concentration–response curve to phenylephrine (0.1 nM–3 μM) was constructed in each ring. Rings were then randomly allocated to one or more of the following protocols: cumulative concentration–response curve to phenylephrine (0.1 nM–3 μM) in the presence or absence of L-NAME (100 μM, 20 min pre-incubation); cumulative concentration–response curve to acetylcholine (0.1 nM–100 μM); cumulative concentration–response curve to glyceryl trinitrate (0.1 nM–1 μM). Relaxant responses to acetylcholine and glyceryl trinitrate were obtained following pre-constriction to the half-maximal effective concentration (EC_{50}) of phenylephrine (10–300 nM) and relaxation expressed as a percentage of this preconstriction (thoracic aorta: 0.7–0.9 g; carotid artery: 0.3–0.5 g).

2.4. Materials

Acetylcholine chloride, phenylephrine hydrochloride, N^G-nitro-L-arginine methyl ester (L-NAME) were obtained

Table 1
Summary of tail cuff blood pressure values and responses to KCl in endothelium-containing (E^+) and endothelium-denuded ($E-$) rings of thoracic aorta (TA) and carotid artery (CA) from 12-week-old male and female SHRSP and age-matched WKY rats

	WKY				SHRSP			
	Male		Female		Male		Female	
B.P. (mm Hg)	132 ± 3		123 ± 2		176 ± 5[a,c]		143 ± 4[b]	
KCl (g tension)	E^+	$E-$	E^+	$E-$	E^+	$E-$	E^+	$E-$
TA	0.82 ± 0.03	0.74 ± 0.04	0.73 ± 0.02	0.72 ± 0.03	0.88 ± 0.03	0.83 ± 0.04	0.79 ± 0.03	0.83 ± 0.03
CA	0.46 ± 0.02	0.42 ± 0.02	0.43 ± 0.02	0.44 ± 0.02	0.44 ± 0.02	0.36 ± 0.02	0.40 ± 0.02	0.42 ± 0.02

[a] Denotes a difference due to sex ($P < 0.001$); [b] and [c] a difference due to strain; ($P < 0.01$ and $P < 0.001$, respectively) ANOVA, followed by Bonferroni analysis. Data are mean ± S.E.M., $n \geq 6$ animals.

from Sigma (Poole, Dorset). Glyceryl trinitrate was obtained from NAPP Laboratories (Cambridge, UK). Euthetal (sodium pentobarbitone) was obtained from Rhone Merieux. All drugs were dissolved in 0.9% saline.

2.5. Statistical analysis

Results are expressed as the mean ± S.E.M. for n separate experiments. EC_{50} and log EC_{50} values were calcu-

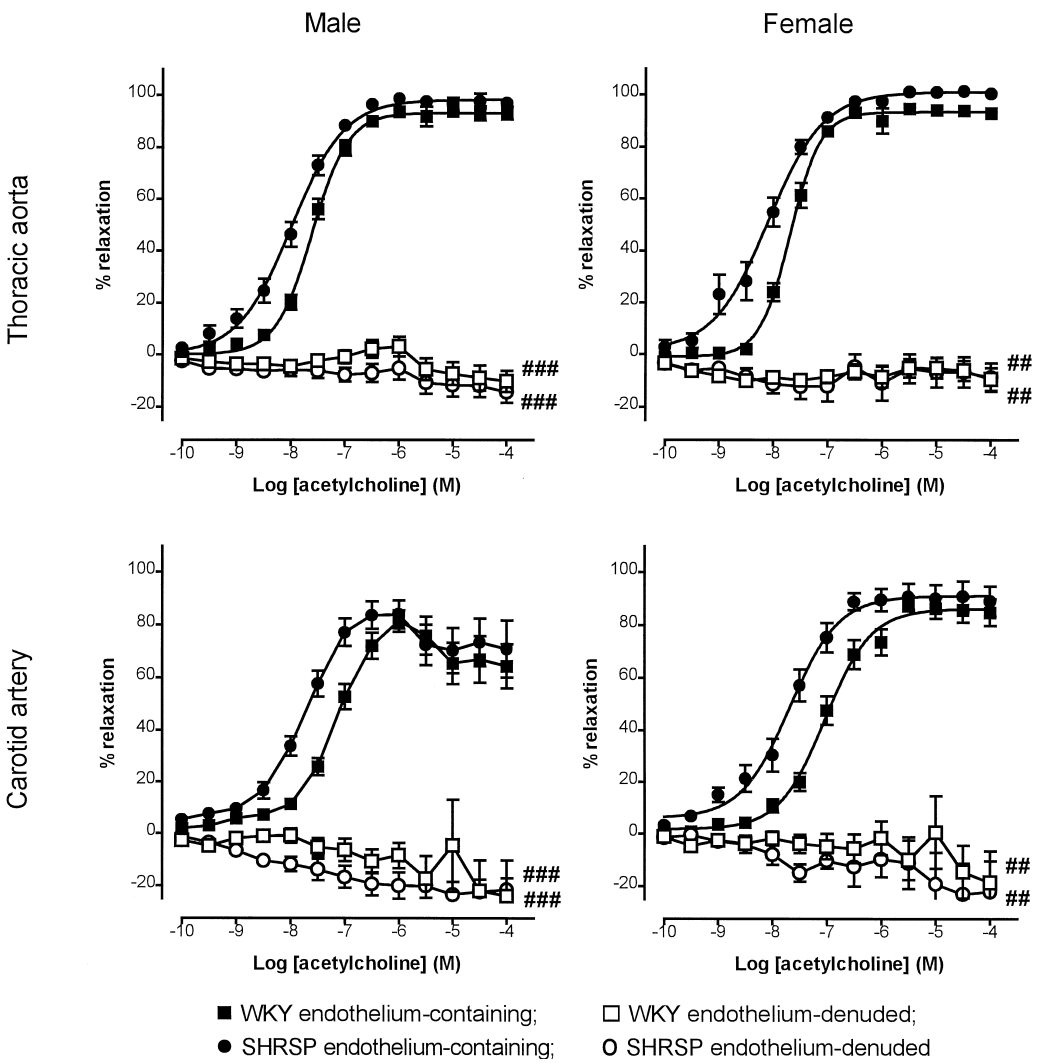

Fig. 1. Concentration–response curves showing relaxation to acetylcholine in rings of thoracic aorta and carotid artery from male and female SHRSP and age-matched WKY rats. ### Denotes a difference due to endothelial denudation ($P < 0.001$ ANOVA, followed by Bonferroni analysis). Data are mean ± S.E.M., $n \geq 6$ animals.

Table 2
Summary of pD_2 values to phenylephrine (PE), acetylcholine (ACh) and glyceryl trinitrate (GTN) in endothelium-containing (E +) and endothelium-denuded (E −) rings of thoracic aorta (TA) and carotid artery (CA) from 12-week-old male and female SHRSP and age-matched WKY rats

		WKY				SHRSP			
		Male		Female		Male		Female	
		E +	E −	E +	E −	E +	E −	E +	E −
TA	PE	6.99 ± 0.06	7.85 ± 0.04[f]	6.86 ± 0.06	7.78 ± 0.03[f]	7.51 ± 0.07[d]	8.02 ± 0.06[f]	7.12 ± 0.07[a,c]	7.96 ± 0.12[f]
	ACh	7.52 ± 0.07		7.60 ± 0.06		7.98 ± 0.12[c]		8.20 ± 0.14[d]	
	GTN	8.18 ± 0.16	8.52 ± 0.24	7.66 ± 0.06[a]	8.42 ± 0.13[e]	8.29 ± 0.20	8.30 ± 0.24	8.36 ± 0.17[c]	8.27 ± 0.10
CA	PE	6.87 ± 0.04	7.60 ± 0.09[f]	6.79 ± 0.06	7.59 ± 0.07[f]	7.30 ± 0.07[d]	7.87 ± 0.08[f]	7.25 ± 0.09[d]	7.97 ± 0.11[b,f]
	ACh	7.05 ± 0.08		6.67 ± 0.13		7.56 ± 0.12[b]		7.53 ± 0.18[d]	
	GTN	7.47 ± 0.24	7.89 ± 0.18	7.34 ± 0.15	8.13 ± 0.08[f]	7.68 ± 0.09	7.76 ± 0.07	7.60 ± 0.03	7.79 ± 0.15

[a]Denotes a difference due to sex ($P < 0.05$); [b,c and d]a difference due to strain ($P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively), [e and f]a difference due to endothelial denudation ($P < 0.01$ and $P < 0.001$, respectively) ANOVA, followed by Bonferroni analysis. Data are mean ± S.E.M., $n \geq 6$ animals.

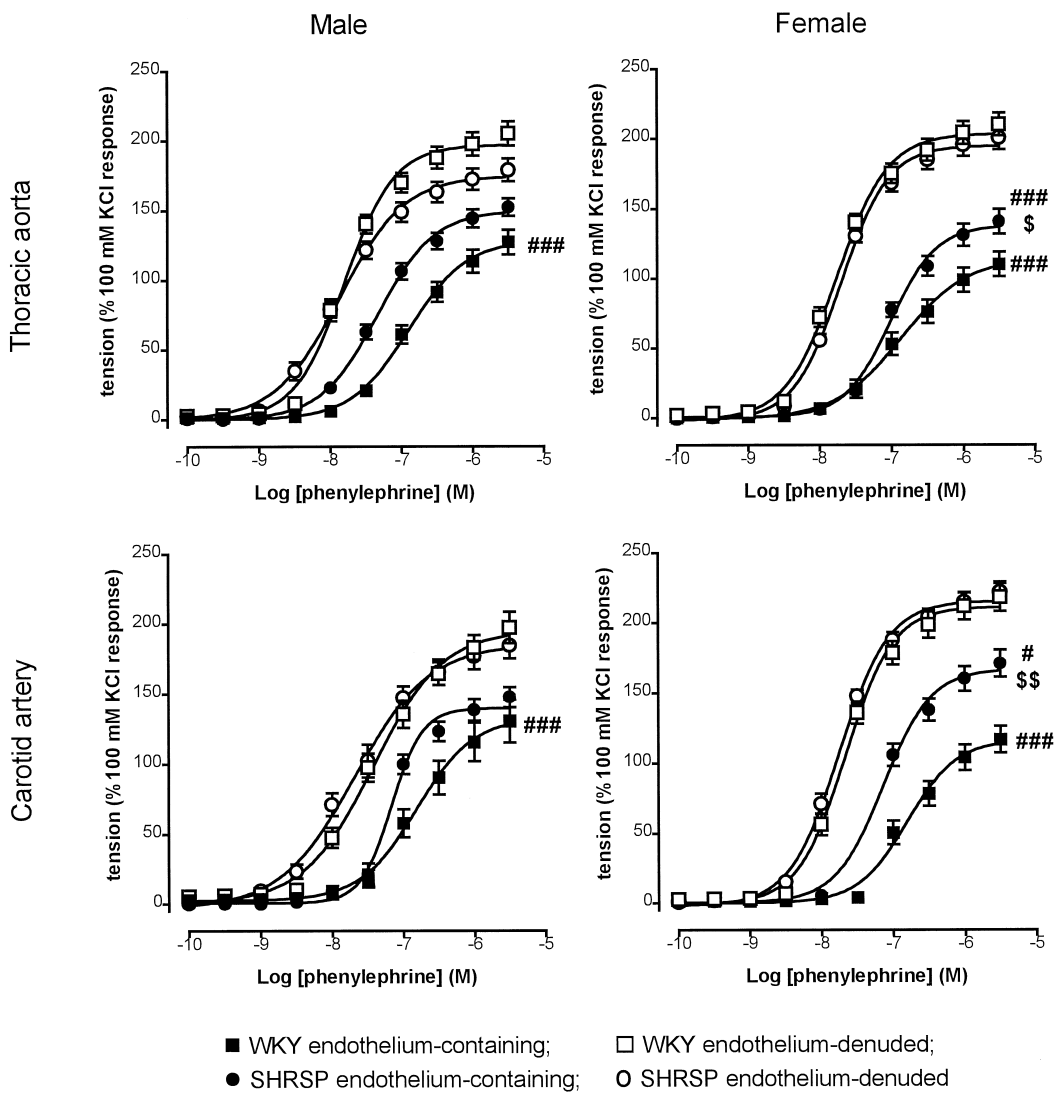

Fig. 2. Concentration–response curves showing contraction to phenylephrine in rings of thoracic aorta and carotid artery from male and female SHRSP and age-matched WKY rats. [$]Denotes a difference in maximum response due to strain; [#]a difference due to endothelial denudation ([$]denotes $P < 0.05$, [$$]denotes $P < 0.01$, [###] or [$$$]denotes $P < 0.001$ ANOVA, followed by Bonferroni analysis). Data are mean ± S.E.M., $n \geq 6$ animals.

lated by a computer-based curve fitting program (Prism, GraphPad). Multiple comparisons were made by one-way analysis of variance (ANOVA). If $P < 0.05$, individual pairs of means were then compared by the Bonferroni multiple comparisons test.

3. Results

3.1. Baseline parameters

3.1.1. Blood pressure

Systolic blood pressure values are given in Table 1. Male and female SHRSP rats were both hypertensive when compared to their respective WKY controls, although this was significantly more pronounced in the males. There was no significant difference in the blood pressures of male and female WKY rats.

3.1.2. KCl contraction

The contractile response to 100 mM KCl in rings of thoracic aorta and carotid artery are given in Table 1. For each of the two blood vessels there was no significant difference in the magnitude of response due to sex, strain or endothelial denudation.

3.2. Effects of agonist-stimulated nitric oxide

The effects of agonist-stimulated nitric oxide were assessed by constructing cumulative concentration–response curves to acetylcholine (Fig. 1). Whilst no difference in maximum response was observed, there was a significantly higher sensitivity (Table 2) to acetylcholine in rings of thoracic aorta and carotid artery from SHRSP when compared to WKY rats. There was no difference in response in the two sexes. Endothelial-denudation completely abolished the relaxation induced by acetylcholine in all rings.

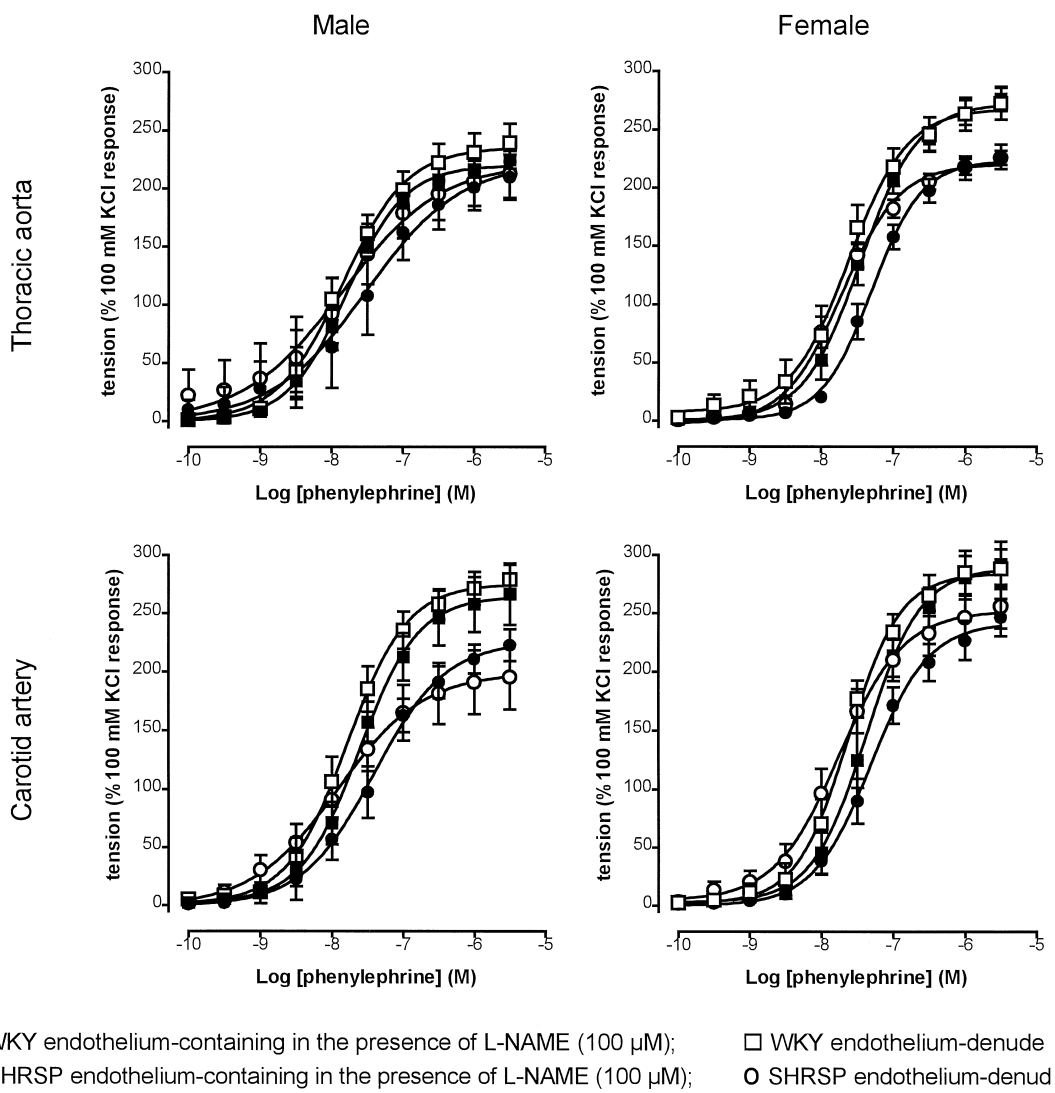

Fig. 3. Concentration–response curves showing the effect of L-NAME (100 μM, 20 min pre-incubation) on contraction to phenylephrine in rings of thoracic aorta and carotid artery from male and female SHRSP and age-matched WKY rats. Data are mean ± S.E.M., $n \geq 6$ animals.

A slight contractile response to acetylcholine was seen in endothelium-denuded rings of aorta and carotid artery, however, no difference due to strain or sex was observed.

3.3. Effects of basal nitric oxide

The effects of basal nitric oxide were assessed by examining the influence of endothelial denudation and L-NAME (100 μM) on phenylephrine-induced contraction and also by assessing the influence of endothelial denudation on relaxation to glyceryl trinitrate.

The presence of the endothelium caused a significant decrease in sensitivity (Table 2) to phenylephrine in rings of both thoracic aorta and carotid artery in both strains and sexes. The sensitivity to phenylephrine was higher in endothelium-containing rings from SHRSP than from WKY rats in both males and females. The maximum response to phenylephrine in both thoracic aorta and carotid artery was also significantly enhanced following endothelial denudation (Fig. 2) in female WKY and SHRSP rats and also in male WKY rats but not in male SHRSP rats. In females the maximum response to phenylephrine in both thoracic aorta and carotid artery was significantly higher in endothelium-containing rings from SHRSP than WKY rats but this was not the case in males. Following endothelial denudation of thoracic aorta and carotid artery there was no significant alteration in either sensitivity or maximum response in the two strains and sexes.

L-NAME increased the sensitivity and maximum response to phenylephrine in endothelium-containing rings of thoracic aorta and carotid artery (Fig. 3) to levels seen in endothelium-denuded rings. L-NAME had no effect on endothelium-denuded vessels of both strains and sexes (data not shown).

The presence of the endothelium produced a significant decrease in sensitivity to glyceryl trinitrate in both thoracic

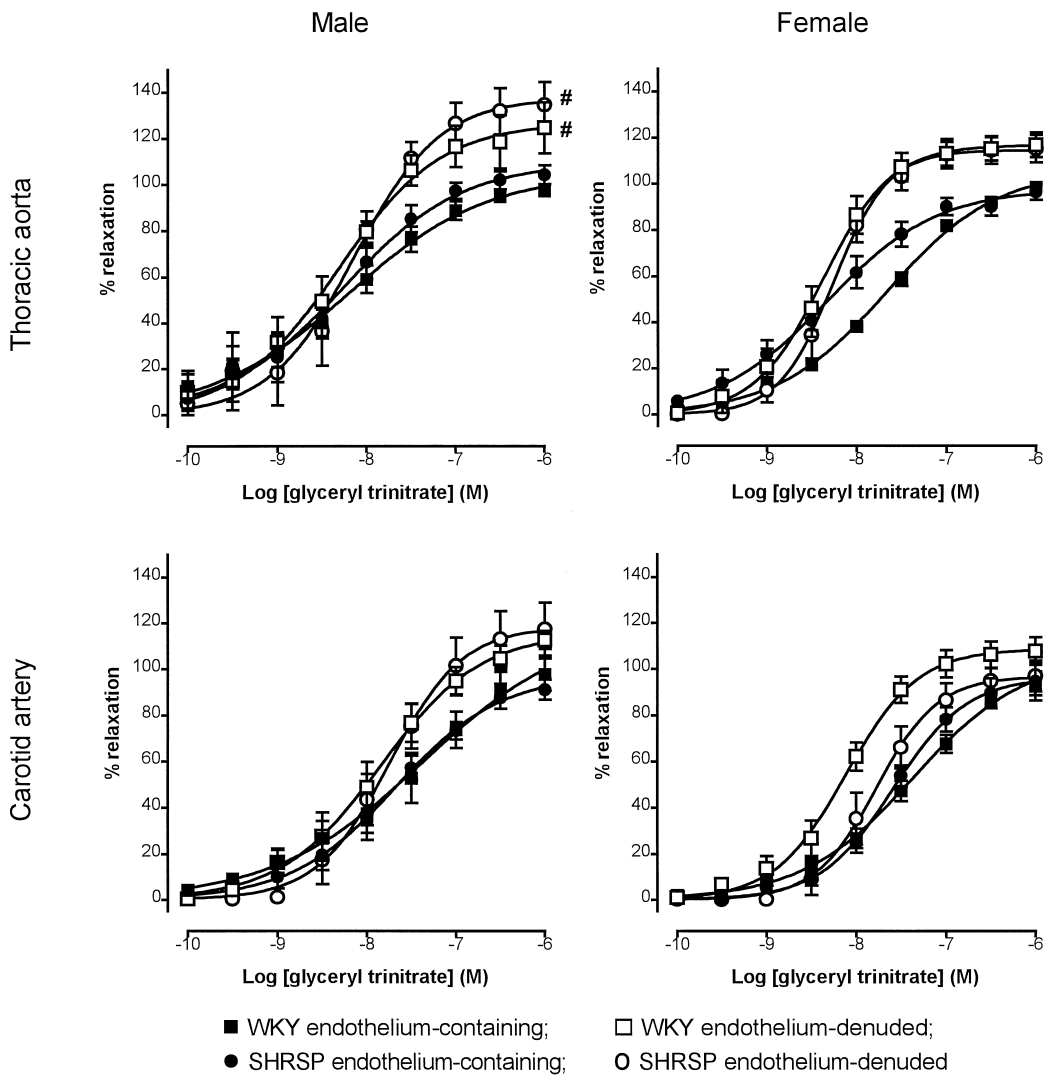

Fig. 4. Concentration–response curves showing relaxation to glyceryl trinitrate in rings of thoracic aorta and carotid artery from male and female SHRSP and age-matched WKY rats. # Denotes a difference in maximum response due to endothelial denudation ($P < 0.05$ ANOVA, followed by Bonferroni analysis). Data are mean ± S.E.M., $n \geq 6$ animals.

aorta and carotid artery from female WKY rats (Fig. 4, Table 2). This effect was not seen in female SHRSP, nor in males of either strain. The presence of the endothelium produced a significant depression of the maximum response to glyceryl trinitrate in rings of thoracic aorta from male rats of both strains.

4. Discussion

We have previously reported a reduced ability of a nitric oxide synthase inhibitor to elevate tone induced by a single concentration of phenylephrine (EC_{20}) in thoracic aorta from 16-week-old SHRSP when compared with those from age-matched normotensive control WKY rats (McIntyre et al., 1997), suggesting a deficiency of basal nitric oxide in the hypertensive strain. We also reported that endothelium-dependent relaxation was attenuated in thoracic aorta from the hypertensive strain. In the present study younger animals (12 weeks old) were studied. In these animals acetylcholine-induced endothelium-dependent relaxation in thoracic aorta was found to be enhanced five-fold in both male and female SHRSP when compared with those from age- and sex-matched normotensive control WKY rats. This is in accordance with previous studies of endothelium-dependent relaxation in thoracic aorta from young (8 weeks old) SHRSP (Onda et al., 1994; Tomita et al., 1995). In addition, we found acetylcholine-induced endothelium-dependent relaxation was similarly enhanced in the carotid artery from the SHRSP when compared with those from WKY rats, illustrating that this effect is not simply localised to the aorta.

The changes in endothelium-dependent relaxation in the SHRSP appear to depend on developmental age; studies in older animals have shown a progressive decline relative to the WKY control, as age increases (Onda et al., 1994; Matsuda et al., 1995; Tomita et al., 1995; Kitazono et al., 1996). The early enhancement of endothelium-dependent relaxation seen in this study at 12 weeks may be a compensatory mechanism in response to the developing hypertension. Indeed we have previously reported an increase in mRNA for eNOS in thoracic aorta from 12-week-old SHRSP (Kerr et al., 1999) and higher levels of eNOS activity in endothelial cells harvested from aortae of SHRSP when compared to the same cells obtained from WKY controls (McIntyre et al., 1997). However, superoxide anion generation is also greater in the isolated aorta of the SHRSP than of the WKY rat (Kerr et al., 1999). Superoxide anion interacts with nitric oxide to produce peroxynitrite (Beckman et al., 1994), a powerful oxidant which, if produced over prolonged periods of time, may result in vascular damage and consequently contribute to the decline in endothelium-dependent relaxation seen with increasing age in SHRSP rats.

In contrast to the clear enhancement of agonist (acetylcholine)-stimulated nitric oxide-induced relaxation, we found a depression in the effects of basal nitric oxide in the SHRSP rat. Specifically, the ability of basal nitric oxide to depress contractile responses to phenylephrine was found to be reduced in both the thoracic aorta and carotid artery from SHRSP when compared with those from WKY rats. This effect was particularly marked in the male rats, where basal levels of nitric oxide are lower (present study and McIntyre et al., 1997). The loss of basal nitric oxide was demonstrated by endothelial denudation and with the use of L-NAME to inhibit eNOS. L-NAME completely abolished the endothelium-dependent depression of phenylephrine-induced tone, thus confirming that this was entirely due to the basal effects of nitric oxide. A reduction in basal nitric oxide has previously been demonstrated in thoracic aorta from older (16 weeks old) SHRSP rats where clear evidence of impaired acetylcholine-induced relaxation was seen (Osugi et al., 1990; Matsuda et al., 1995; McIntyre et al., 1997). By contrast, this is the first observation of reduced basal nitric oxide in SHRSP at an age where agonist-stimulated nitric oxide responses are not impaired, but are indeed enhanced. Furthermore, this is the first report that this is not simply localised to the aorta, since identical data were obtained in the carotid artery. The loss of the effects of basal nitric oxide at a time when agonist-stimulated responses remain enhanced may reflect the greater susceptibility of basal than of agonist-stimulated nitric oxide to destruction by superoxide anion (Mian and Martin, 1995).

Further evidence that the effects of basal nitric oxide are reduced in the SHRSP rat was obtained when we assessed the endothelium-dependent depression of vasodilator responses to the nitric oxide donor, glyceryl trinitrate (Shirasaki and Su, 1985; Moncada et al., 1991b; Maruyama and Maruyama, 1994). In the aorta and carotid artery of male rats removal of the endothelium only had a slight enhancing effect on the concentration–response curve to glyceryl trinitrate, with no difference observed between vessels from SHRSP and WKY rats. In contrast, removal of the endothelium in vessels from female rats resulted in a clear enhancement of the sensitivity to glyceryl trinitrate, an effect which was markedly impaired in vessels from the female SHRSP. The fact that endothelial denudation had a lesser effect on the glyceryl trinitrate response in males than females presumably reflects the lower levels of basal nitric oxide found in male rats. As a consequence of this, any strain difference in the males would have been hard to observe. Previous studies have reported that relaxant responses to another nitric oxide donor sodium nitroprusside are similar in aorta from SHRSP and WKY rats, however these studies have either examined only males (Tesfamariam and Halpern, 1988; Miyata et al., 1990; Onda et al., 1994) or alternatively have not assessed the effects of endothelial denudation in order to remove the powerful modulating action of basal nitric oxide (McIntyre et al., 1997).

Thus, in summary, this study has shown that the effects of basal nitric oxide are impaired in the SHRSP rat at an

age when agonist-stimulated nitric oxide is actually enhanced when compared with control WKY rats. This impairment may be related to the greater susceptibility of basal nitric oxide to destruction by superoxide anion, which is known to be produced in excess in this (Kerr et al., 1999) and other models of hypertension (Bouloumie et al., 1997; Laursen et al., 1997).

Acknowledgements

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