ELSEVIER

European Journal of Pharmacology 358 (1998) 31–40

ejp

Characterization of endothelium-dependent relaxations in mesenteries from transgenic hypertensive rats

Michael D. Randall *, Julie E. March

School of Biomedical Sciences, E-Floor, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK

Received 22 January 1998; revised 3 August 1998; accepted 5 August 1998

Abstract

Endothelial dysfunction has been reported to be a feature of hypertension. We have investigated the relative contributions of nitric oxide (NO) and the endothelium-derived hyperpolarizing factor (EDHF) to endothelium-dependent relaxations in isolated mesenteries from (mREN-2)-27 transgenic hypertensive (TGH) rats and their normotensive controls (Hannover Sprague–Dawley). Relaxation to the endothelium-dependent relaxant, carbachol, was unimpaired in mesenteries from TGH rats compared to the Hannover Sprague–Dawley controls. Inhibition of NO synthase (with 100 μM N^{ω}-nitro-L-arginine methyl ester) had greater inhibitory effects against these relaxations in the mesenteries from Hannover Sprague–Dawley compared to TGH. Inhibition of EDHF activity with high K^+ also had greater inhibitory effects against endothelium-dependent relaxations in the mesenteries from the Hannover Sprague–Dawley compared to TGH. The present results show that, although endothelium-dependent relaxation is unimpaired in mesenteries from TGH rats, there are differences in the relative contributions of NO and EDHF, such that inhibition of either NO or EDHF alone in TGH mesenteries has less impact compared to Hannover Sprague–Dawley. It is suggested that the recently identified reciprocal relationship between NO and EDHF is upregulated in the mesenteries from the TGH rats.

Keywords: Endothelium; Hypertension; mRen-2 Transgenic hypertensive rat; Nitric oxide (NO); EDHF (endothelium-derived hyperpolarizing factor); Mesenteric arterial bed

1. Introduction

In several animal models of hypertension agonist-induced endothelium-dependent relaxations are impaired in conduit blood vessels (Winquist et al., 1984; Lockette et al., 1986; Lüscher and Vanhoutte, 1986; Mayhan et al., 1987; Miller et al., 1987). Impairment of endothelium-dependent relaxations has been ascribed to decreased release of endothelium-derived autacoids (Miller et al., 1987), to reduced responsiveness of vascular smooth muscle to these substances (Lockette et al., 1986) or to production of endothelium-derived vasoconstrictor prostanoids (Lüscher and Vanhoutte, 1986; Noll et al., 1997).

The situation is less clear in resistance beds, where both impaired (Watt and Thurston, 1989; Fu-Xiang et al., 1992; Bennett et al., 1996) and normal (Cachofeiro and Nasjletti, 1991; Randall et al., 1991; Dunn and Gardiner, 1995; McCulloch and Randall, 1997) endothelium-dependent relaxations have been reported. Furthermore, in conscious hypertensive Brattleboro rats, the regional haemodynamic responses to endothelium-dependent relaxants, acting via both nitric oxide (NO)-dependent and -independent pathways, are similar to those in normotensive controls (Gardiner et al., 1994). Whilst it has been suggested that impairment depends on the experimental conditions (Li and Bukoski, 1993). In terms of biochemical evidence, Kelm et al. (1995) reported that, in isolated perfused hearts from spontaneously hypertensive rats (SHR), agonist-evoked NO levels were similar to normotensive controls and basal NO production was enhanced. Furthermore, Nava et al. (1995) have reported that the constitutive NO synthase is upregulated in cardiac endothelial cells from SHR. More recently, Tschudi et al. (1996) have reported that in mesenteric vessels from SHR, NO release is normal but its decomposition is enhanced by augmented superoxide anion release.

* Corresponding author. Tel.: +44-115-9709484; Fax: +44-115-9709259; E-mail: michael.randall@nottingham.ac.uk

0014-2999/98/$19.00
PII: S0014-2999(98)00584-6

In addition to NO, another relaxant, the endothelium-derived hyperpolarizing factor (EDHF; Chen et al., 1988; Taylor and Weston, 1988; Garland et al., 1995) also contributes to endothelium-dependent relaxations and appears of greatest importance in resistance vessels (Garland et al., 1995). Further, it appears that NO and EDHF have a reciprocal relationship, whereby EDHF activity becomes upregulated on loss of NO production (Bauersachs et al., 1996; McCulloch et al., 1997). Although, many studies have determined endothelium-dependent vasodilator function in a variety of animal models of hypertension, very few have addressed the relative functions of NO and EDHF. In this respect, Vargas et al. (1995) have reported that, in the isolated kidney from rats rendered hypertensive through chronic NO synthase inhibition, the EDHF-mediated component appears upregulated as a counter to the loss of NO. Conversely, in isolated kidneys, Hayakawa et al. (1993) have reported that EDHF activity is impaired in SHRs, whilst the release of NO is normal.

Given the sparsity of the literature concerning the relative contributions of NO and EDHF to agonist-induced endothelium-dependent relaxations we have now investigated their relative roles in the isolated mesenteric arterial bed from transgenic (mREN-2)27 hypertensive rats (TGH) (Mullins et al., 1990). TGH rats have been produced by insertion of the mouse renin-2 gene into the rat genome, producing cardiovascular changes due to a defined genetic alteration (Mullins et al., 1990). Hannover Sprague–Dawley rats have been used as the appropriate normotensive controls. Endothelium-dependent relaxations have been evoked by carbachol in both TGH and Hannover Sprague–Dawley mesenteries and the role of NO investigated by NO synthase inhibition with N^{ω}-nitro-L-arginine methyl ester (L-NAME). The contribution of EDHF has been assessed by perfusion with high extracellular K^+ (Adeagbo and Triggle, 1993; McCulloch et al., 1997). As an index of the influence of basal NO, the vasoconstrictor responses to methoxamine have been determined in the presence and absence of L-NAME (Moore et al., 1990; Randall and Griffith, 1991). We have also investigated the vascular responses to the exogenous NO-donor sodium nitroprusside and the K^+-channel activator levcromakalim. In all of the experiments the cyclo-oxygenase inhibitor, indomethacin, was included in the perfusion fluid as Noll et al. (1997) have demonstrated that in TGH rats the endothelium may release vasoconstrictor prostanoids.

2. Materials and methods

2.1. Animals

TGH and Hannover Sprague–Dawley rats were bred in the Biomedical Services Unit, University of Nottingham Medical School, from animals supplied by Dr. J.J. Mullins at the Centre for Genome Research, Edinburgh. Male, heterozygous transgenic hypertensive rats (TGH) (5–8 months old) and age-matched Sprague–Dawley rats (originally from Zentralinstitut für Versuchstierkunde, Hannover) were studied. The heterozygous TGH rats were bred by crossing male, homozygous TGH rats with the control Hannover Sprague–Dawley rats. The homozygous TGH rats were kept on chronic captopril treatment (50 mg l^{-1} in the drinking water; Mullins et al., 1990), but the heterozygous animals used in this study were untreated.

2.2. Measurement of arterial pressure in conscious rats

Ten of the rats were anaesthetized (sodium methohexitone, Brietal, Lilly, Basingstoke, UK; 40–60 mg kg^{-1}, i.p.) and had polyethylene catheters implanted in the abdominal aorta (via the caudal artery), for blood pressure and heart rate recording, and in the right jugular vein for the administration of heparin and anaesthetic (see below). At least 24 h later, measurements of mean arterial blood pressure and heart rate were made in fully conscious, freely moving animals, using a Gould electrostatic recorder (ES 1000 with SP400 preamplifier) transducer amplifier and rate meter (Biotach model 13-4613-65A) (Gould Electronics, Cleveland, OH, USA).

2.3. Preparation of the isolated buffer-perfused superior mesenteric arterial bed

The rats (500–600 g) were anaesthetised with sodium pentobarbitone (60 mg kg^{-1}, i.p., or 40 mg kg^{-1}, i.v., Sagatal, Rhône Mérieux, Harlow, Essex, UK). A midline incision was made, a cannula was inserted into the superior mesenteric artery and the vascular bed was flushed with Krebs–Henseleit solution. The arterial vasculature was dissected away from the intestines, transferred to a jacketed organ bath (37°C) as described previously by Randall and Hiley (1988), and perfused at 5 ml min^{-1} with gassed (95% O_2/5% CO_2) Krebs–Henseleit solution at 37°C (composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2, D-glucose 10), by means of a peristaltic pump (Watson–Marlow 504S). Indomethacin (10 μM) was present throughout the entire experiment

The perfusion pressure in superior mesenteric arterial bed was continuously monitored by means of a pressure transducer coupled to a Maclab 4e recording system (AD Instruments, New South Wales, Australia). Flow was maintained constant (5 ml min^{-1}) and therefore changes in perfusion pressure represented alterations in vascular resistance. At the end of each experiment, the cannula pressure was measured and subtracted from the recorded basal perfusion pressure in order to determine the pressure drop across the bed.

2.4. Experimental protocol

Following a 30-min equilibration period, perfusion pressure was raised by addition of methoxamine (10–30 μM) to the perfusion buffer to achieve a submaximal increase in perfusion pressure of ca. 100 mmHg. The vasorelaxant effects of the endothelium-dependent relaxant carbachol, the NO-donor sodium nitroprusside or the ATP-sensitive K^+-channel activator levcromakalim were independently assessed against the induced tone. The involvement of NO in the endothelium-dependent relaxations to carbachol was determined by repeating the experiments in the presence of the NO synthase inhibitor L-NAME (100 μM). In view of the augmented vasoconstrictor responses in the presence of L-NAME, the concentration of methoxamine used in the experiments was reduced (1–3 μM) to induce an equivalent level of tone. The relaxations to levcromakalim were also repeated in the presence of L-NAME, as we have previously shown that basal NO may influence relaxation to this agent (Randall and Griffith, 1993; McCulloch and Randall, 1996). Indeed, differences between the absence and presence of L-NAME, may act as an indirect marker of basal NO activity.

In order to define the involvement of EDHF the relaxant effects of carbachol were also determined against tone established by perfusion with 60 mM KCl (Adeagbo and Triggle, 1993; McCulloch et al., 1997). The high K^+ buffer was obtained by substituting equimolar amounts of KCl for NaCl. Finally, relaxation was also determined against tone established by high K^+ perfusion in the presence of L-NAME (100 μM). Vasorelaxants were administered close-arterially as bolus doses in random order. Each preparation was used to investigate the effects of up to two of the vasorelaxants under control and experimental conditions.

In order to assess the influence of basal NO, dose–response curves were determined for methoxamine in the absence and then presence of L-NAME (100 μM).

2.5. Data and statistical analysis

All data are presented as mean ± S.E.M. ED_{50} values for vascular responses were obtained from individual dose–response curves as the dose at which the half-maximal response occurred. These variables were determined by fitting the data to the logistic equation:

$$R = \frac{R_{max} A^{n_H}}{ED_{50}^{n_H} + A^{n_H}}$$

where R is the response, A the dose of the agent, R_{max} the maximal response, n_H the slope function and ED_{50} the dose of vasorelaxant giving half the maximal relaxation. The curve fitting was carried out using KaleidaGraph Software (Synergy, Reading, PA, USA). Statistical analysis of the variables was carried out by analysis of variance with Bonferroni's post-hoc test.

2.6. Drugs

All drugs were prepared on the day of the experiment. Levcromakalim (a generous gift from SmithKline Beecham, Surrey) was dissolved in absolute ethanol and diluted in 0.9% saline. Methoxamine, L-NAME, sodium nitroprusside and carbachol (all from Sigma Chemical) were all dissolved at the relevant concentrations in Krebs–Henseleit solution.

3. Results

3.1. Baseline cardiovascular variables in vivo and in vitro

In conscious, unrestrained TGH rats the mean arterial pressure was 165 ± 4 mmHg ($n = 5$) which was significantly ($P < 0.001$) higher than the corresponding value in the Hannover Sprague–Dawley control rats (113 ± 2 mmHg, $n = 5$). In the mesenteries from TGH rats basal perfusion pressure was 26.2 ± 2.0 mmHg ($n = 27$), compared to 17.6 ± 1.1 mmHg ($n = 19$) in mesenteries from Hannover Sprague–Dawley rats ($P < 0.01$). In the presence of methoxamine the levels of induced tone were not significantly different between groups with values of 97.5 ± 9.7 mmHg (Hannover Sprague–Dawley, $n = 13$) and 115 ± 9 mmHg (TGH, $n = 14$).

3.2. Effects of L-NAME on endothelium-dependent vasorelaxation to carbachol

In eight mesenteries from Hannover Sprague–Dawley rats carbachol (5.5 pmol–164 nmol) caused dose-related relaxations of tone described by $ED_{50} = 379 \pm 75$ pmol, $R_{max} = 76.1 \pm 2.7\%$ and $n_H = 0.67 \pm 0.05$ (Fig. 1). In 11 preparations from TGH rats, carbachol was significantly ($P < 0.01$) more potent as a vasorelaxant ($ED_{50} = 161 \pm 22$ pmol), whilst R_{max} was no different (73.3 ± 1.7%) and $n_H = 0.86 \pm 0.07$ (Fig. 1).

Addition of L-NAME (100 μM) to the mesenteries from the Hannover Sprague–Dawley rats decreased both the potency ($ED_{50} = 6.45 \pm 2.2$ nmol, $P < 0.01$) and R_{max} (57.0 ± 2.7%, $P < 0.01$, $n = 6$) with $n_H = 0.65 \pm 0.06$ (Fig. 1). In the TGH preparations the presence of L-NAME also decreased the potency of carbachol ($ED_{50} = 5.84 \pm 2.4$ nmol, $P < 0.01$, $n = 6$) but did not affect the maximum relaxation (80.9 ± 4.9%) (Fig. 1) and $n_H = 0.53 \pm 0.04$.

3.3. Effects of 60 mM KCl on endothelium-dependent relaxations to carbachol

In Hannover Sprague–Dawley mesenteries preconstricted by perfusion with 60 mM KCl relaxation to carbachol was impaired (Fig. 2) to that observed against methoxamine-induced tone reported above with $ED_{50} = 39.7 \pm 6.4$ nmol ($P < 0.001$), $R_{max} = 31.4 \pm 4.8\%$ ($P <$

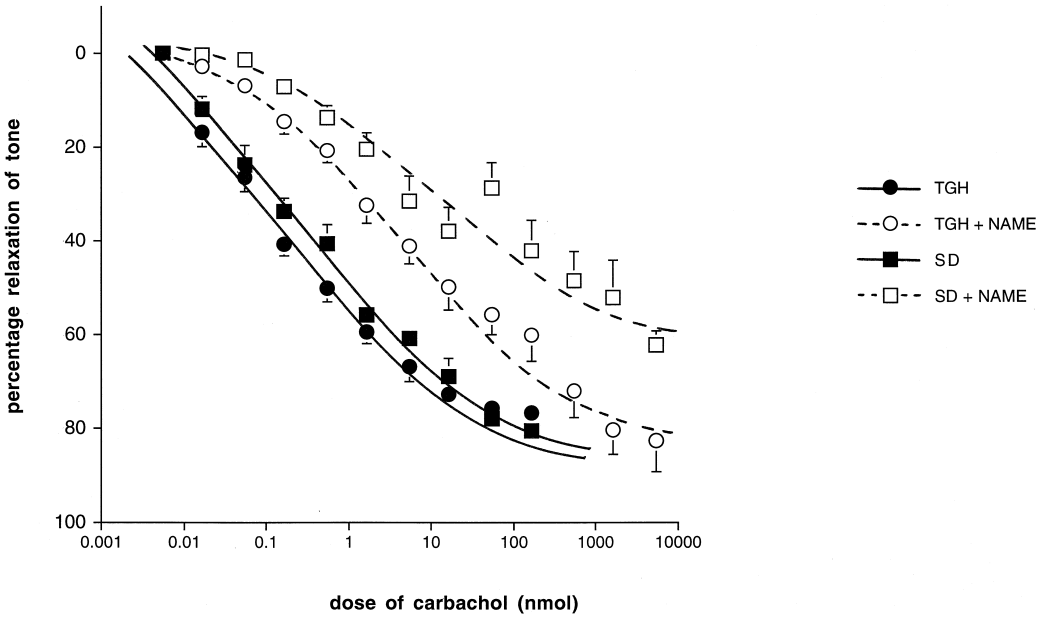

Fig. 1. Vasorelaxant effects of carbachol against methoxamine-induced tone in isolated perfused mesenteric vascular beds from normotensive Sprague–Dawley (SD) controls and mRen-2 transgenic hypertensive (TGH) rats in the absence and presence of L-NAME (100 μM). The data are given as mean ± S.E.M. with vertical bars representing the S.E.M.

0.001) and $n_H = 0.33 \pm 0.09$ ($n = 7$). Although high K^+ perfusion also resulted in significantly ($P < 0.05$) reduced relaxations to carbachol in the TGH mesenteries ($n = 7$) compared to those observed against methoxamine-induced tone, the effects were less pronounced than in the mesenteries from the Hannover Sprague–Dawley rats with $ED_{50} = 3.86 \pm 1.65$ nmol and $R_{max} = 46.1 \pm 3.3\%$ (which were significantly different from the corresponding value in the Hannover Sprague–Dawley rats, with $P < 0.001$ and $P < 0.05$, respectively) and $n_H = 0.55 \pm 0.07$ (Fig. 2).

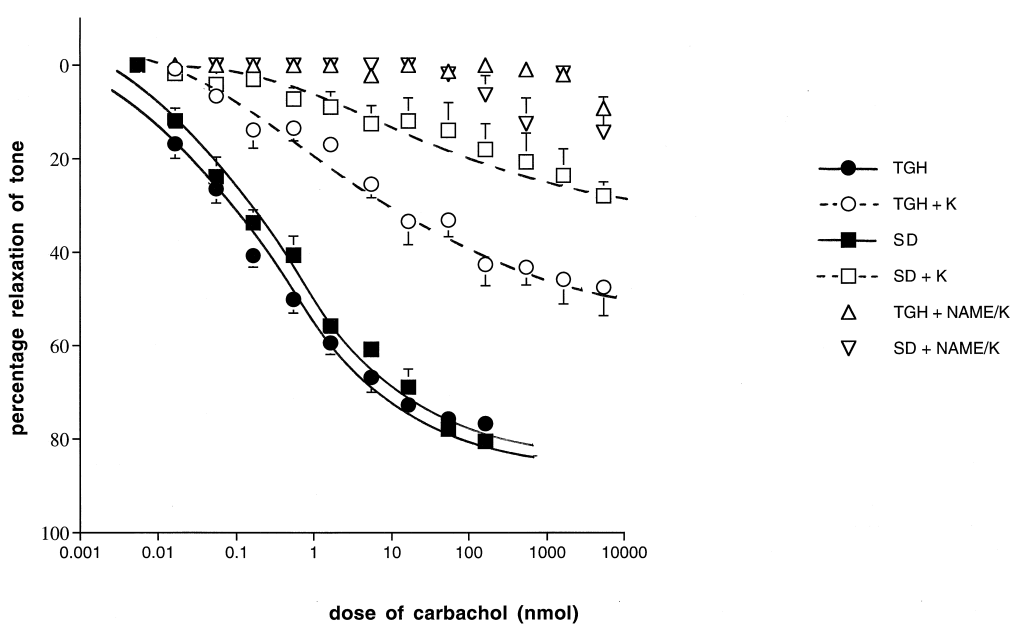

Fig. 2. Vasorelaxant effects of carbachol in isolated perfused mesenteric vascular beds from normotensive Sprague–Dawley (SD) controls and mRen-2 transgenic hypertensive (TGH) rats against methoxamine-induced tone (data taken from Fig. 1) and tone raised by high K^+ (60 mM) in the absence and presence of L-NAME (100 μM). The data are given as mean ± S.E.M. with vertical bars representing the S.E.M.

3.4. Effects of L-NAME plus 60 mM KCl on endothelium-dependent relaxations to carbachol

Fig. 2 shows that in the presence of both high K^+ and L-NAME (100 μM) the endothelium-dependent relaxations to carbachol were abolished.

3.5. Relaxant effects of sodium nitroprusside

In methoxamine-preconstricted preparations from Hannover Sprague–Dawley rats sodium nitroprusside induced dose-related relaxations of tone ($ED_{50} = 3.18 \pm 0.87$ nmol, $R_{max} = 72.9 \pm 3.8\%$, and $n_H = 0.59 \pm 0.05$, $n = 5$) (Fig. 3). Under identical conditions sodium nitroprusside was significantly ($P < 0.05$) more potent (5.7-fold) in the mesenteries from the TGH rats ($ED_{50} = 554 \pm 97$ pmol), whilst the maximum reactivity was not different ($R_{max} = 74.8 \pm 2.4\%$) and $n_H = 0.72 \pm 0.06$ ($n = 5$).

In both groups the addition of L-NAME (100 μM) increased the relaxant potency of sodium nitroprusside (Fig. 3). In the Hannover Sprague–Dawley rats the $ED_{50} = 61.4 \pm 13.8$ pmol ($P < 0.05$) and the R_{max} was significantly ($P < 0.01$) increased to $97.3 \pm 4.6\%$; $n_H = 1.11 \pm 0.17$ ($n = 4$). In the TGH rats the ED_{50} was also significantly ($P < 0.001$) reduced to 27.2 ± 3.7 pmol, while R_{max} was significantly ($P < 0.01$) increased to $86.8 \pm 2.0\%$ and $n_H = 0.99 \pm 0.21$ ($n = 5$).

3.6. Relaxant effects of levcromakalim

In methoxamine-preconstricted preparations from Hannover Sprague–Dawley rats the K^+-channel activator levcromakalim induced dose-related relaxations of tone ($ED_{50} = 7.31 \pm 2.94$ nmol, $R_{max} = 62.8 \pm 8.7\%$, and $n_H = 1.35 \pm 0.25$, $n = 7$) (Fig. 4). In the mesenteries from the TGH rats levcromakalim was equipotent as a vasorelaxant ($ED_{50} = 4.55 \pm 0.56$ nmol, $n = 5$) but the maximum relaxation was significantly ($P < 0.05$) greater ($R_{max} = 87.4 \pm 2.5\%$) with $n_H = 1.44 \pm 0.19$ (Fig. 4). In both cases the relaxant effects of levcromakalim were abolished in the presence of high K^+ ($n = 5–7$).

In the presence of 100 μM L-NAME, levcromakalim caused comparable relaxations between strains (Fig. 4). Specifically, in mesenteries from Hannover Sprague–Dawley the responses were now enhanced compared to those in the absence of L-NAME, with R_{max} increased significantly ($P < 0.01$) to $95.0 \pm 2.0\%$ ($n = 5$) and was now not different to the R_{max} in the TGH group in the presence of L-NAME ($95.9 \pm 1.8\%$, $n = 5$). The ED_{50} values were now 1.73 ± 0.20 nmol (Hannover Sprague–Dawley) and 1.78 ± 0.33 nmol (TGH). The n_H values were 1.59 ± 0.13 (Hannover Sprague–Dawley) and 1.42 ± 0.19 (TGH).

3.7. Vasoconstrictor responses to methoxamine

Fig. 5 shows that in four preparations from the Hannover Sprague–Dawley rats methoxamine induced dose-related pressor effects ($ED_{50} = 65.7 \pm 10.8$ nmol, $R_{max} = 162 \pm 8$ mmHg and $n_H = 0.86 \pm 0.04$). In the mesenteric preparations from the TGH rats methoxamine was equipotent ($ED_{50} = 73.0 \pm 18.1$ nmol) but was significantly ($P < 0.05$) more reactive ($R_{max} = 233 \pm 20$ mmHg, $n = 5$) with $n_H = 1.09 \pm 0.01$ (Fig. 5).

The presence of L-NAME (100 μM) caused leftward shifts in the dose–response curves in both groups (Fig. 5).

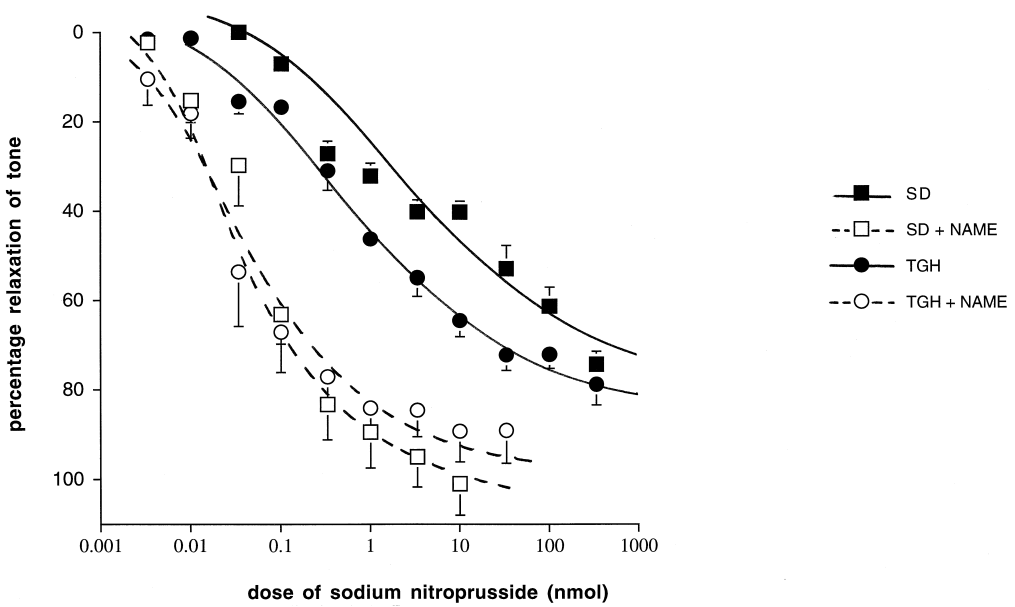

Fig. 3. Vasorelaxant effects of sodium nitroprusside against methoxamine-induced tone in isolated perfused mesenteric vascular beds from normotensive Sprague–Dawley (SD) controls and mRen-2 transgenic hypertensive (TGH) rats in the absence and presence of L-NAME (100 μM). The data are given as mean ± S.E.M. with vertical bars representing the S.E.M.

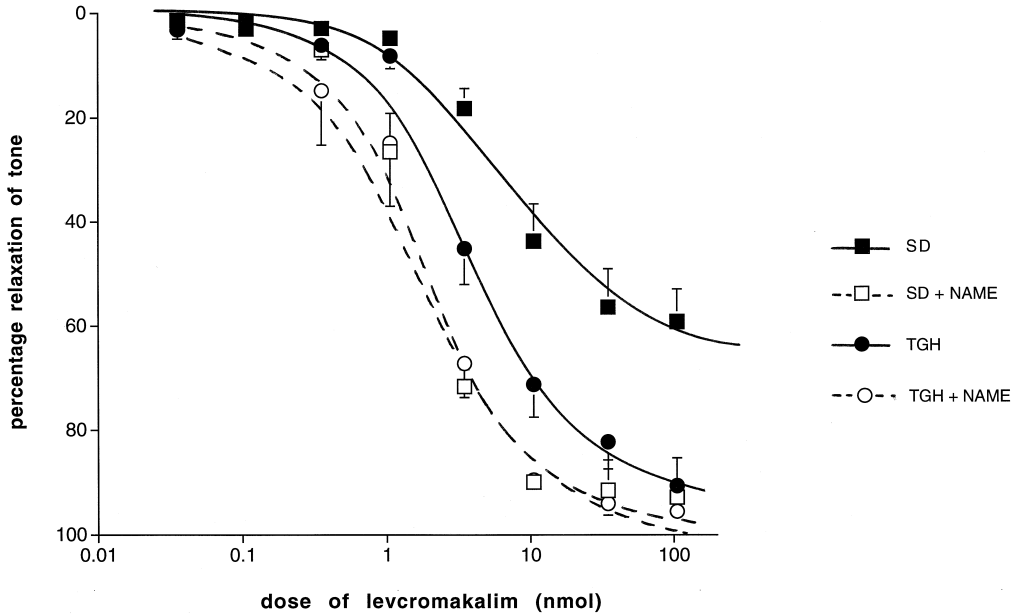

Fig. 4. Vasorelaxant effects of levcromakalim against methoxamine-induced tone in isolated perfused mesenteric vascular beds from normotensive Sprague–Dawley (SD) controls and mRen-2 transgenic hypertensive (TGH) rats in the absence and presence of L-NAME (100 μM). The data are given as mean ± S.E.M. with vertical bars representing the S.E.M.

In the Hannover Sprague-Dawley mesenteries, the ED_{50} for methoxamine was significantly ($P < 0.01$) decreased to a value of 7.8 ± 1.3 nmol, whilst R_{max} was unaltered (146 ± 5 mmHg, $n = 4$) and $n_H = 1.05 \pm 0.08$. In the TGH preparations, the ED_{50} was also significantly ($P < 0.05$) reduced to 14.5 ± 2.4 nmol and R_{max} was unaltered (247 ± 15 mmHg, $n = 5$) and $n_H = 1.03 \pm 0.09$. Accordingly, in the presence of L-NAME, methoxamine was significantly ($P < 0.05$) less potent in TGH compared to Hannover Sprague–Dawley. Therefore, the NO synthase

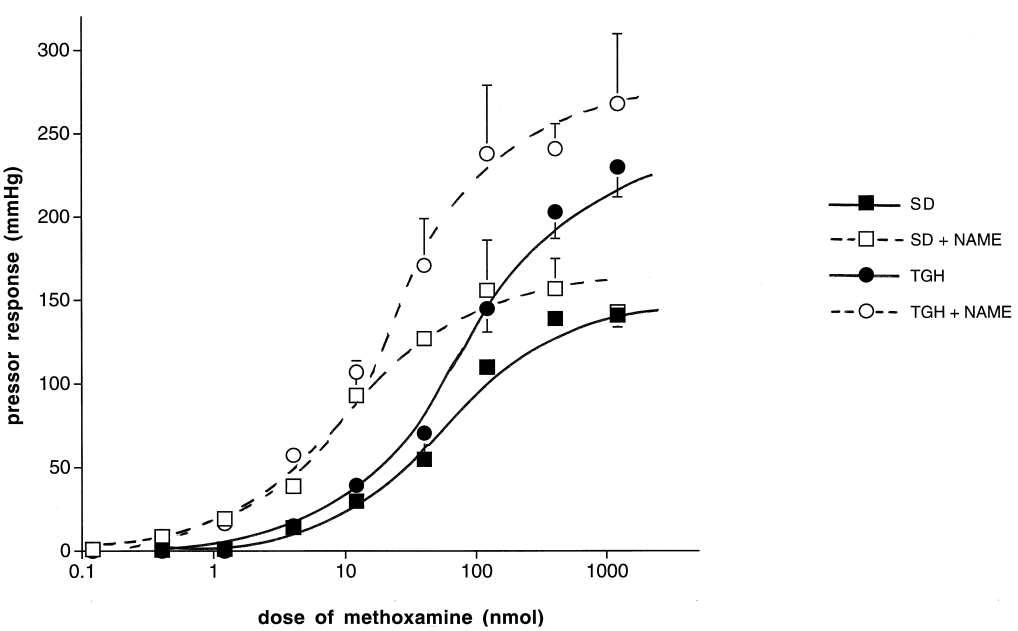

Fig. 5. Pressor effects of methoxamine in isolated perfused mesenteric vascular beds from normotensive Sprague–Dawley (SD) controls and mRen-2 transgenic hypertensive (TGH) rats in the absence and presence of L-NAME (100 μM). The data are given as mean ± S.E.M. with vertical bars representing the S.E.M.

inhibitor had caused a greater ($P < 0.05$) leftward shift in the responses to methoxamine in the Hannover Sprague–Dawley preparations (8.4-fold vs. 5.0-fold).

4. Discussion

In the present investigation, we have clearly shown that endothelium-dependent relaxations are not impaired in the mesenteric resistance vasculature of rats with mRen-2 transgenic hypertension. However, differences have been identified in the relative contributions of, or relationship between, NO and EDHF to these endothelium-dependent relaxations.

The first observation that endothelium-dependent relaxations in this resistance bed were not impaired, contrasts the findings in isolated conduit vessels (Winquist et al., 1984; Lockette et al., 1986; Lüscher and Vanhoutte, 1986; Mayhan et al., 1987; Miller et al., 1987) but is in full agreement with findings in resistance beds of other rat models of hypertension (Cachofeiro and Nasjletti, 1991; Randall et al., 1991; Dunn and Gardiner, 1995; McCulloch and Randall, 1997). In isolated aortae and coronary arteries from TGH rats the preservation of endothelium-dependent relaxations has also been reported by others (Arribas et al., 1994; Tschudi et al., 1994). Similarly, in conscious unrestrained TGH rats, endothelial function, as assessed by cardiovascular responses to endothelium-dependent relaxants, also appears largely unimpaired (Gardiner et al., 1998).

The principal aim of the present study was to investigate the relative contributions of NO and EDHF to agonist-induced endothelium-dependent relaxations. In this respect, it was found that blockade of NO synthase with L-NAME opposed the endothelium-dependent responses to carbachol, however, in both groups a substantial L-NAME -resistant component was revealed. This NO-independent component has been widely observed in preparations from normotensive rats (see McCulloch et al., 1997) and is generally ascribed to EDHF. In contrast, in isolated coronary arteries from both TGH and Hannover Sprague–Dawley rats endothelium-dependent relaxations are entirely NO-mediated (Tschudi et al., 1994). In the present study, inhibition of NO synthase had a greater impact against the relaxations in the Hannover Sprague–Dawley mesenteries compared to the TGH preparations, such that in the former there was depression of the maximum response and also a greater rightward shift in the dose–response curve. Superficially, this observation may be taken to suggest that NO contributes less to endothelium-dependent relaxations in the TGH compared to Hannover Sprague–Dawley. The relationship between NO and EDHF has recently been shown to be dynamic, with EDHF activity becoming upregulated on loss of basal or co-released NO due to a cGMP-mediated modulation at the smooth muscle (McCulloch et al., 1997) or an interaction at the endothelium (Bauersachs et al., 1996). This reciprocal relationship may perhaps explain the present findings, with EDHF being more effective at compensating for the loss of NO in the TGHs.

It is noteworthy that, in mesenteric vessels from the TGHs, the endothelium also releases vasoconstrictor prostanoids (Noll et al., 1997). Although their involvement in the present study was excluded by the addition of indomethacin to the perfusion fluid, it is of interest that following NO synthase blockade Noll et al. reported that this system was also upregulated, perhaps suggesting that basal NO may modulate their release, analogous to the suppression of EDHF activity (McCulloch et al., 1997).

Further experiments determined the relative contributions of EDHF, in the presence of basal NO, in both strains. In these studies, high K^+ was used to inhibit the EDHF-mediated component of the endothelium-dependent relaxations and it appeared that EDHF contributed more to endothelium-dependent relaxations in the Hannover Sprague–Dawley preparations compared to the TGH vascular beds. It is noteworthy that the inhibitory effects of high K^+ were substantially greater in the Hannover Sprague–Dawley preparations compared to previous findings in normotensive Wistar rats (McCulloch et al., 1997) and may reflect strain differences. The present observations are in contrast to the above findings that NO appeared to play a greater role in the relaxations in the Hannover Sprague–Dawley mesenteries. Taken together these findings indicate that in the TGH mesenteries the endothelium-dependent relaxations are more resistant to individual inhibition of either NO or EDHF, whilst the total abolition of responses in the combined presence of L-NAME and high K^+ suggests that these are the only mediators. A reciprocal relationship between EDHF and NO has recently been identified (Bauersachs et al., 1996; McCulloch et al., 1997), where EDHF activity is upregulated on loss of NO. It is, therefore, possible that the present observations may reflect an upregulation of these compensatory mechanisms in the TGH mesenteries. Indeed the high K^+ experiments were necessarily carried out in the presence of basal NO. It is conceivable that differences in basal NO or alterations in mechanisms underlying the NO:EDHF interaction may have altered the relationship between these autacoids.

An alternative explanation for the observation that high K^+ had greater inhibitory effects in the Hannover Sprague–Dawley preparations could be that the NO-mediated component may have also been partly sensitive to high K^+ and that this is impaired in the TGHs. There is some evidence that a component of NO-mediated vasorelaxation may occur via K-channel activation (Tare et al., 1990). In our experience, high K^+ does indeed, albeit modestly, have inhibitory effects on NO-mediated vasorelaxation (Randall et al., 1997). In the context of endothelium-dependent relaxations Van de Voorde et al. (1992) have found in aortae from renal hypertensive rats that

endothelium-dependent hyperpolarization is impaired. Although the authors did not determine whether the hyperpolarization was NO or EDHF-mediated, it is possible that such a deficit could perhaps explain the smaller inhibitory effects of K^+ in the TGH preparations. However, if NO was acting by an additional K^+-channel mediated mechanism in the Hannover Sprague–Dawley preparations and not in the TGHs, it is surprising that relaxation to sodium nitroprusside was enhanced in the mesenteries from the TGHs. Furthermore, in the TGH preparation relaxation to the hyperpolarizing agent levcromakalim was unimpaired in the present study.

To investigate the vascular smooth muscle responsiveness to a hyperpolarizing agent, the K_{ATP}-channel activator levcromakalim was used. In both strains levcromakalim caused relaxations, but was more reactive in the TGH preparations. This finding contrasts that in the mesenteric arteries from SHRs, were vasorelaxation to levcromakalim is substantially impaired (Ohya et al., 1996) but is in general agreement with the findings of Van de Voorde et al. (1992). The present observation may suggest that the vascular smooth muscle in vessels from TGH rats may be more responsive to hyperpolarizing agents, perhaps due to differences in resting membrane or the depolarised potentials in response to methoxamine. Certainly in aortae from renal hypertensive rats the resting membrane potential is relatively depolarized compared to controls (Van de Voorde et al., 1992).

Basal NO modulates the activity of K^+-channel activators, whose responses are reduced in its presence (Randall and Griffith, 1993; McCulloch and Randall, 1996). In the present study the apparent reduced reactivity in Hannover Sprague–Dawley controls compared to TGH was reversed by inhibition of NO synthesis with L-NAME. Such that in the absence of basal NO the responses to levcromakalim were identical between strains. This would suggest that basal NO has greater influence on K^+-channel activator responses in mesenteries from Hannover Sprague–Dawley controls. This would suggest that either basal NO is reduced in TGH or that NO was more effective at modulating hyperpolarizing responses in preparations from Hannover Sprague–Dawley compared to TGH. Either way this observation is compatible with the finding that EDHF responses were more prominent in TGH mesenteries compared to Hannover Sprague–Dawley controls.

Vasorelaxation to the NO-donor sodium nitroprusside also differed between strains. In this respect sodium nitroprusside was more potent in mesenteric vascular beds from TGH rats compared to the Hannover Sprague–Dawley controls. In vivo the hypotensive effect of sodium nitroprusside also appears greater in TGH compared to Hannover Sprague–Dawley rats (Gardiner et al., 1998), although this was not observed by Chung et al. (1991). In the present study addition of L-NAME increased both the potency and maximum reactivity in both TGH and Hannover Sprague–Dawley preparations, and indeed in the presence of NO synthase blockade the responses were similar between groups. This indicates that in both strains, vascular smooth muscle responsiveness to NO is the same and does not account for the differences observed. Accordingly, this finding contrasts the observation that guanylate cyclase activity may be impaired in hypertension (Lüscher et al., 1988). The finding that removal of basal NO augments sodium nitroprusside-induced relaxations is widely recognised as reflecting the interaction of endogenous and exogenous NO at the guanylate cyclase (Pohl and Busse, 1987; Moncada et al., 1991). In the case of the TGH vascular beds, L-NAME only increased the potency of sodium nitroprusside by 20.3-fold, compared to the 51.8-fold increase in the Hannover Sprague–Dawley control preparations. Hence basal NO appears to have less influence in the TGH preparations, providing further, indirect evidence, that basal NO may be impaired in this form of hypertension.

In view of the clear differences in the vascular responses between the normotensive and hypertensive strains which may reflect reduced basal NO activity in the former, the basal influence of NO was determined, albeit indirectly, by investigating the effects of NO synthase blockade on vasoconstrictor responses. The α_1-adrenoceptor agonist methoxamine constricted mesenteric vascular beds from both strains with equal potency but was more reactive in the TGH vessels. This observation is consistent with many others on vessels from hypertensive animals (see Folkow et al., 1973). In the context of mesenteric vessels from TGH rats Dunn and Gardiner (1997) have shown that these vessels, although not exhibiting remodelling, have increased vascular smooth muscle content which could potentially contribute toward the increased reactivity. In the present study, inhibition of NO synthase in both strains increased the sensitivity, but not the reactivity, to methoxamine, indicating that basal NO modulated the vasoconstrictor responses in both. This augmentation is generally regarded as a measure of the influence of basal NO (Moore et al., 1990; Randall and Griffith, 1991) and in the present study L-NAME caused an 8.4-fold leftward shift dose–response curve in the Hannover Sprague–Dawley vessels compared to a 5-fold shift in the TGH vascular beds. These results, therefore, point to basal NO having a slightly reduced influence on vascular tone in the TGH. These findings are in accord with indirect observations above with levcromakalim and sodium nitroprusside, which also provided indirect evidence for the decreased influence of NO in TGH.

Consistent with decreased basal NO in TGH, Tschudi et al. (1994) reported that contractile responses to L-NAME were severely impaired in coronary vessels from mature TGH rats, suggesting loss of basal NO. By contrast, in TGHs (Moriguchi et al., 1994; Gardiner et al., 1998) and SHRs (Minami et al., 1995) in vivo inhibition of NO synthase has greater pressor effects relative to the normotensive controls. This would perhaps indicate enhanced

basal NO release, although Gardiner et al. (1998) point out that this observation may not be a straightforward index of NO activity in vivo.

In summary, although endothelium-dependent relaxations appear unimpaired in the mesenteric vasculature of TGH rats, there are differences in the relative contributions of NO and EDHF. These differences are not straightforward in that it appears in the TGH mesenteries that inhibition of either NO production or EDHF activity alone has less impact compared to that observed in the Hannover Sprague–Dawley vascular beds. One possible explanation for this apparent difference is that the reciprocal relationship between NO and EDHF (McCulloch et al., 1997) is upregulated in TGH. In addition to these changes in the relationship between NO and EDHF, there also appears to be a slight reduction of basal NO activity in TGH. Indeed, it is possible that this reduction in basal NO may account for the apparent alteration the NO:EDHF relationship.

Acknowledgements

This study was funded by a project grant from the British Heart Foundation (PG94060) and the University of Nottingham. We thank Professor S.M. Gardiner for constructive comments on the MS and Dr. J. Mullins for providing breeding pairs Hannover Sprague–Dawley and TGH rats.

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.

Arribas, S., Sanchez-Ferrer, C.F., Peiro, C., Ponte, A., Salaices, M., Marin, J., 1994. Functional vascular renin–angiotensin system in hypertensive rats for the mouse renin gene *Ren-2*. Gen. Pharmacol. 25, 1163–1170.

Bauersachs, J., Popp, R., Hecker, M., Sauer, E., Fleming, I., Busse, R., 1996. Nitric oxide attenuates the release of endothelium-derived hyperpolarising factor. Circulation 94, 3341–3347.

Bennett, M.A., Hillier, C., Thurston, H., 1996. Endothelium-dependent relaxation in resistance arteries from spontaneously hypertensive rats: effects of long term treatment with perindopril, quinapril, hydralazine and amlodipine. J. Hypertens. 14, 389–397.

Cachofeiro, V., Nasjletti, A., 1991. Increased vascular responsiveness to bradykinin in kidneys of spontaneously hypertensive rats. Hypertension 18, 683–688.

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.

Chung, O., Schips, T., Qadri, F., Ganten, D., Mullins, J., Unger, T., 1991. The transgenic mRen-2 rat: cardiovascular characterization of a novel hypertensive animal model. J. Hypertens. 9 (Suppl. 6), S430.

Dunn, W.R., Gardiner, S.M., 1995. Structural and functional properties of isolated, pressurized, mesenteric resistance arteries from a vasopressin-deficient rat model of genetic hypertension. Hypertension 26, 390–396.

Dunn, W.R., Gardiner, S.M., 1997. Differential alteration in vascular structure of resistance arteries isolated from the cerebral and mesenteric vascular beds of transgenic [(m*Ren*-2)27, hypertensive rats. Hypertension 29, 1140–1147.

Folkow, B., Hallbåck, M., Lundgren, Y., Sivertsson, R., Weiss, L., 1973. Importance of adaptive changes in vascular design for establishment of primary hypertension, studied in man and in spontaneously hypertensive rats. Circ. Res. 32 (Suppl. I), I2–I16.

Fu-Xiang, D., Jameson, M., Skopec, J., Diederich, A., Diederich, D., 1992. Endothelial dysfunction of resistance arteries of spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 20, S190–S192, (Suppl. 12).

Gardiner, S.M., March, J.E., Kemp, P.A., Bennett, T., 1994. Resting cardiovascular status and vasodilator function in a vasopressin-deficient, hypertensive strain of rat. J. Hypertens. 12, 1217–1224.

Gardiner, S.M., March, J.E., Kemp, P.A., Bennett, T., 1998. The contribution of nitric oxide to cardiovascular status and responses to vasodilators in conscious, hypertensive, transgenic ((mRen-2)27) rats. Br. J. Pharmacol. 124, 299–306.

Garland, C.J., Plane, F., Kemp, B.K., Cocks, T.M., 1995. Endothelium-dependent hyperpolarization: a role in the control of vascular tone. Trends Pharmacol. Sci. 16, 23–30.

Hayakawa, H., Hirata, Y., Suzuki, E., Sugimoto, T., Matasuoka, H., Kirkuchi, K., Nagano, T., Hirobe, M., Sugimoto, T., 1993. Mechanisms for altered endothelium-dependent vasorelaxation in isolated kidneys from experimental hypertensive rats. Am. J. Physiol. 264, H1535–H1541.

Kelm, M., Feelisch, M., Krebber, T., Deussen, A., Motz, W., Strauer, B.E., 1995. Role of NO in the regulation of coronary vascular tone in hearts from hypertensive rats. Hypertension 25, 186–193.

Li, J., Bukoski, R.D., 1993. Endothelium-dependent relaxation of hypertensive resistance arteries is not impaired under all conditions. Circ. Res. 72, 290–296.

Lockette, W., Otsuka, Y., Carretero, O., 1986. The loss of the endothelium-dependent vascular relaxation in hypertension. Hypertension 8 (Suppl. II), II61–II66.

Lüscher, T.F., Vanhoutte, P.M., 1986. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. Hypertension 8, 344–348.

Lüscher, T.F., Diederich, D., Weber, E., Vanhoutte, P.M., Buhler, F.R., 1988. Endothelium-dependent responses in carotid and renal arteries of normotensive and hypertensive rats. Hypertension 11, 573–578.

Mayhan, W.G., Faraci, F.M., Heistad, D.D., 1987. Impairment of endothelium-dependent-responses of cerebral arteries in chronic hypertension. Am. J. Physiol. 253, H1435–H1440.

McCulloch, A.I., Randall, M.D., 1996. Modulation of vasorelaxant responses to potassium channel openers by basal nitric oxide in the rat isolated superior mesenteric arterial bed. Br. J. Pharmacol. 117, 859–866.

McCulloch, A.I., Randall, M.D., 1997. Relative contributions of nitric oxide and EDHF to endothelium-dependent relaxations in isolated perfused mesenteric bed from normotensive and hypertensive Brattleboro rats. Br. J. Pharmacol. 122, 121P.

McCulloch, A.I., Bottrill, F.E., Randall, M.D., Hiley, C.R., 1997. Characterization and modulation of EDHF-mediated relaxations in the rat isolated superior mesenteric arterial bed. Br. J. Pharmacol. 120, 1431–1438.

Miller, M.J.S., Pinto, A., Mullane, K.M., 1987. Impaired endothelium-dependent relaxations of rabbits subjected to aortic coarctation hypertension. Hypertension 10, 164–170.

Minami, N., Imaj, Y., Hashimoto, J.-I., Abe, K., 1995. Contribution of vascular nitric oxide to basal blood pressure in conscious spontaneously hypertensive rats and normotensive Wistar Kyoto rats. Clin. Sci. 89, 177–182.

Moncada, S., Rees, D.D., Schulz, R., Palmer, R.M.J., 1991. Development and mechanism of a specific supersensitivity to nitrovasodilators

following inhibition of vascular nitric oxide synthesis in vivo. Proc. Natl. Acad. Sci. USA 88, 2166–2170.

Moore, P.K., Al-Swayeth, O.A., Chong, N.W.S., Evans, R.A., Gibson, A., 1990. L-N^G-nitro arginine (L-NOARG), a novel L-arginine-reversible inhibitor of endothelium-dependent vasodilatation in vitro. Br. J. Pharmacol. 99, 408–412.

Moriguchi, A., Brosnihan, K.B., Kumagai, H., Ganten, D., Ferrario, C.M., 1994. Mechanisms of hypertension in transgenic rats expressing the mouse *Ren-2* gene. Am. J. Physiol. 266, R1273–R1279.

Mullins, J.J., Peters, J., Ganten, D., 1990. Fulminant hypertension in transgenic rats harbouring the mouse *Ren-2* gene. Nature 344, 541–544.

Nava, E., Noll, G., Lüscher, T.F., 1995. Increased activity of constitutive nitric oxide synthase in cardiac endothelium in spontaneous hypertension. Circulation 91, 2310–2313.

Noll, G., Lang, M.G., Tschudi, M.R., Ganten, D., Lüscher, T.F., 1997. Endothelial vasoconstrictor prostanoids modulate contractions to acetylcholine and ANG II in Ren-2 rats. Am. J. Physiol. 272, H493–H500.

Ohya, Y., Setoguchi, M., Fujii, K., Nagao, T., Abe, I., Fujishima, M., 1996. Impaired action of levcromakalim on ATP-sensitive K^+ channels in mesenteric artery cells from spontaneously hypertensive rats. Hypertension 27, 1234–1239.

Pohl, U., Busse, R., 1987. Endothelium-derived relaxant factor inhibits effects of nitrocompounds in isolated arteries. Am. J. Physiol. 252, H307–H313.

Randall, M.D., Griffith, T.M., 1991. Differential effects of L-arginine on the inhibition by N^G-nitro-L-arginine methyl ester of basal and agonist-stimulated EDRF activity. Br. J. Pharmacol. 104, 743–749.

Randall, M.D., Griffith, T.M., 1993. Modulation of vasodilatation to levcromakalim by hypoxia and EDRF in the rabbit isolated ear: a comparison with pinacidil, sodium nitroprusside and verapamil. Br. J. Pharmacol. 109, 386–393.

Randall, M.D., Hiley, C.R., 1988. Effect of phenobarbitone pretreatment upon endothelium-dependent relaxation to acetylcholine in rat superior mesenteric arterial bed. Br. J. Pharmacol. 94, 977–983.

Randall, M.D., Thomas, G.R., Hiley, C.R., 1991. Effect of destruction of the vascular endothelium upon pressure/flow relations and endothelium-dependent vasodilatation in resistance beds of spontaneously hypertensive rats. Clin. Sci. 80, 463–469.

Randall, M.D., McCulloch, A.I., Kendall, D.A., 1997. Comparative pharmacology of endothelium-derived hyperpolarizing factor and anandamide in rat isolated mesentery. Eur. J. Pharmacol. 333, 191–197.

Tare, M., Parkintgton, H.C., Coleman, H.A., Neild, T.O., Dusting, G.J., 1990. Hyperpolarization and relaxation of arterial smooth muscle caused by nitric oxide derived from the endothelium. Nature 346, 69–71.

Taylor, S.G., Weston, A.H., 1988. Endothelium-derived hyperpolarizing factor: a new endogenous inhibitor from vascular endothelium. Trends Pharmacol. Sci. 9, 272–274.

Tschudi, M.R., Noll, G., Arnet, U., Novosel, D., Ganten, D., Lüscher, T.F., 1994. Alterations in coronary artery vascular reactivity of hypertensive Ren-2 transgenic rats. Circulation 89, 2780–2786.

Tschudi, M.R., Mesaros, S., Lüscher, T.F., Malinski, T., 1996. Direct in situ measurements of nitric oxide in mesenteric resistance arteries. Hypertension 27, 32–35.

Van de Voorde, J., Vanheel, B., Leusen, I., 1992. Endothelium-dependent relaxation and hyperpolarization in aorta from control and renal hypertensive rats. Circ. Res. 70, 1–8.

Vargas, F., Osuna, A., Fernandez-Rivas, A., 1995. Vascular reactivity and flow-pressure curve in isolated kidneys from rats with *N*-nitro-L-arginine ester-induced hypertension. J. Hypertens. 14, 373–379.

Watt, P.A.C., Thurston, H., 1989. Endothelium-dependent relaxation in resistance vessels from spontaneously hypertensive rats. J. Hypertens. 7, 661–666.

Winquist, R.J., Bunting, P.B., Baslin, E.P., Wallace, A.A., 1984. Decreased endothelium-dependent relaxation in New Zealand genetic hypertensive rats. J. Hypertens. 2, 541–545.