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Unaltered endothelium-dependent modulation of contraction in the pulmonary artery of hypertensive rats

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Abstract

Involvement of endothelium-derived nitric oxide (EDNO) in α-adrenoceptor agonist-induced contractile responses was studied in isolated pulmonary arteries from Wistar Kyoto rats (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP). In the presence of propranolol, noradrenaline-induced contraction was potentiated by endothelium removal or by N^G-nitro-L-arginine (L-NOARG). The magnitude of the potentiation was independent of the noradrenaline concentration. L-NOARG also shifted the concentration–response curves for phenylephrine and methoxamine to the left and upward. Contractile responses to 2-amino-5,6,7,8,-tetrahydro-6-ethyl-4*H*-oxazolo-(5,4-*d*)-azepine-dihydrochloride (BHT-933) and 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK-14304) were augmented by L-NOARG in a concentration-dependent manner. There were no differences in the effects of L-NOARG on the contractile responses to α-adrenoceptor agonists between the preparations from WKY and SHRSP. Endothelium-dependent relaxation in response to acetylcholine was not impaired in the preparations from SHRSP when compared with those from WKY. These observations suggest that the contractile responses to the α_1-adrenoceptor agonists were depressed mainly by basally released EDNO, while the responses to the α_2-adrenoceptor agonists were depressed mainly by EDNO released in response to α_2-adrenoceptor stimulation. The comparable influence of the endothelium on the α-adrenoceptor agonist-induced contractions in the pulmonary arteries from WKY and SHRSP, which were markedly different from other arteries, could be explained by the unaltered endothelium-dependent relaxation in the preparations from SHRSP.

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

1. Introduction

The vascular endothelium controls contractile responses of vascular smooth muscles to agonists by releasing factors such as endothelium-derived relaxing factor (EDRF), contracting factor (EDCF) and hyperpolarizing factor (EDHF)(Vanhoutte, 1989; Vanhoutte et al., 1986; Cohen and Vanhoutte, 1995). Among these factors, the depression by EDRF (Furchgott, 1984) is most remarkable and has been studied most extensively.

EDRF is a nitric oxide (NO) that is synthesized from L-arginine by the action of a specific enzyme (Marletta, 1993). The synthesis of NO can be blocked by compounds such as N^G-monomethyl-L-arginine (L-NMMA; Palmer et al., 1988), N^G-nitro-L-arginine (L-NOARG; Ishii et al., 1990; Moore et al., 1990) or N^G-nitro-L-arginine methylester (L-NAME; Rees et al., 1990).

We have previously reported that the contractile responses of the rat aorta to α-adrenoceptor agonists are depressed by the endothelium (Kaneko and Sunano, 1993; Matsuda et al., 1995). The depression is brought about mainly by endothelium-derived nitric oxide (EDNO) in response to the stimulation of α_2-adrenoceptor in the endothelium. The depressive effect of the endothelium is impaired in preparations from stroke-prone spontaneously hypertensive rats (SHRSP) as compared with that of preparations from normotensive Wistar Kyoto rats (WKY) (Matsuda et al., 1995). This may be due to altered endothelial function, since endothelium-dependent relaxation has been reported to be impaired in blood vessels of hyperten-

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sive rats, including SHR and SHRSP (Sunano et al., 1989; Shimamura et al., 1991; Vanhoutte and Boulanger, 1995). The impairment may be brought about by changes in endothelium function due to sustained hypertension, since it is prevented by antihypertensive treatment (Clozel et al., 1990; Shimamura et al., 1991; Sunano et al., 1992; Dohi et al., 1994; Takase et al., 1996).

It has been reported that blood pressure is much lower in pulmonary arteries of rats than in systemic arteries (Aharinejad et al., 1996). This suggested that the endothelium-dependent relaxation elicited by acetylcholine, and thus, the endothelium-dependent depression of contraction may not be altered in the pulmonary arteries of hypertensive rats. The present study was performed to investigate and compare the influence of the endothelium, especially EDNO, on contractile responses to α-adrenoceptor agonists in the pulmonary arteries of WKY and SHRSP.

2. Materials and methods

2.1. Animals and measurement of blood pressure

Normotensive WKY and SHRSP were originally obtained from Dr. Okamoto (Okamoto et al., 1974) and bred successively in our animal facility. The animals were kept under controlled conditions of 22°C and 50% humidity and under a 12-h light–dark cycle. Normal chow (Funabashi SP, Chiba, Japan) and tap water were given ad libitum. The systemic blood pressure of the rats was measured by the tail cuff method using the Rat-Tail Manometer–Tachometer System (Natsume, KN-210, Tokyo, Japan). Prior to the measurement, the rats were warmed at 40°C for 10 min to allow precise measurement of blood pressure.

2.2. Preparations and solutions

The rats at the age of 16–17 weeks were exsanguinated from the vena cava under ether anesthesia, and perfused via the left ventricle with 200 ml of a modified Tyrode's solution (warmed at 37°C). Left main pulmonary arteries were excised and adhesive fat and connective tissues were removed. Arterial ring preparations 1 mm in width were made from these left main pulmonary arteries under a dissecting microscope, taking care not to damage the endothelium. In some preparations, the endothelium was removed by rubbing the inner surface of the vascular lumen with a soft rubber band. Two thin tungsten wires (30 μm in diameter) were inserted into the lumen of the preparation. One tungsten wire was fixed to a holding rod and the other was connected to a mechano-electronic transducer (Minebea, UL-10GR, Nagano, Japan).

The preparation was immersed in an organ bath (10 ml) filled with a modified Tyrode's solution of the following composition (mM): NaCl, 137; KCl, 5.4; $CaCl_2$, 2.0; $MgCl_2$, 1.0; $NaHCO_3$, 11.9; $NaH_2PO_4 \cdot 2H_2O$, 0.4; glucose, 5.6. The solution was equilibrated with a gas mixture of 95% O_2 and 5% CO_2 (pH of the solution at 37°C was 7.3). High-K^+ Tyrode's solution containing 50 mM K^+ was prepared by replacing NaCl in the solution with equimolar KCl.

2.3. Measurement of tension

Tension changes were measured isometrically under a stretch tension of 8 mN. Preparations were equilibrated in modified Tyrode's solution for at least 60 min. Prior to the experiments, two successive high-K^+-induced contractions were initiated at an interval of 20 min. These procedures were required to obtain constant results during the following experiments. Concentration–response experiments for drugs that induced contraction or relaxation of the preparation were then performed in most of the experiments. In experiments where relaxation was observed, preparations were precontracted with noradrenaline at a concentration that induced submaximal contraction (5×10^{-7} M), and in the stable tonic contraction, acetylcholine or sodium nitroprusside was applied cumulatively. When noradrenaline was applied, calcium disodium ethylenediaminetetraacetate (Ca (II)-EDTA, 26 μM) was added to the Tyrode's solution. In the endothelium-denuded preparations, the complete removal of endothelium was ascertained by observing the absence of relaxation in response to acetylcholine (10^{-5} M) after precontraction with noradrenaline 5×10^{-7} M. After the experiment, the preparations were relaxed completely by application of verapamil (10^{-5} M) and papaverine (10^{-4} M), and all tension changes were measured from this level.

2.4. Drugs

The following drugs were used: noradrenaline ((−)-arterenol bitartrate salt), propranolol hydrochloride, phenylephrine, methoxamine, sodium nitroprusside, L-arginine hydrochloride (Sigma, St. Louis, MO, USA); acetylcholine hydrochloride, indomethacin, Ca (II)-EDTA, verapamil hydrochloride, papaverine hydrochloride (Wako, Osaka, Japan); and L-NOARG (Aldrich Chemical, Milwaukee, WI, USA). 5-Bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK-14304) was provided by Pfizer (Kent, UK). 2-Amino-5,6,7,8,-tetrahydro-6-ethyl-4*H*-oxazolo-(5,4-*d*)-azepine-dihydrochloride (BHT-933) was provided by Boehringer Ingelheim (Ingelheim, Germany). Drug concentrations are expressed as final molar concentrations in the bath solution. All drugs were dissolved in distilled water except indomethacin, which was dissolved in distilled water containing Na_2CO_3 (10^{-2} M) and sonicated before use.

2.5. Data and statistical analysis

The obtained data are expressed as means ± S.E.M. with numbers in parentheses. Tension is expressed as

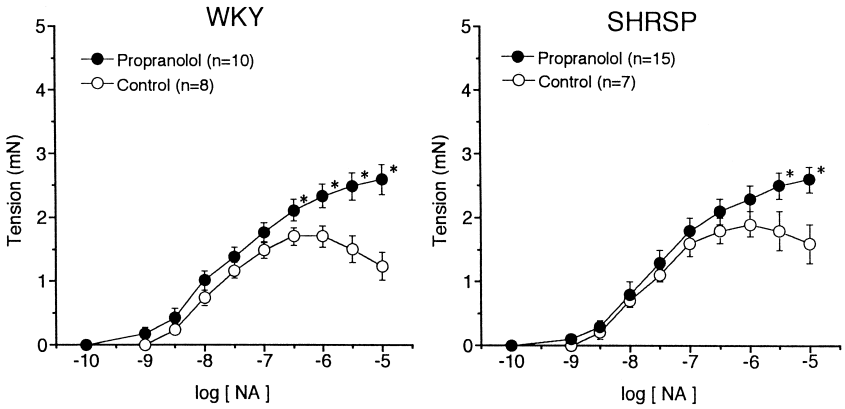

Fig. 1. Concentration–response curves for noradrenaline (NA)-induced contraction and the effect of propranolol in the pulmonary arteries from WKY and SHRSP. Each point represents the mean value of respective experiments (numbers are stated in parentheses). Vertical line attached to each point represents S.E. of the mean. Propranolol 10^{-6} M was applied 10 min prior to the concentration–response experiments. WKY and SHRSP represent the experiment with pulmonary artery preparations from WKY and SHRSP, respectively.

absolute values for contractile response or as percentages of initial pre-constriction for relaxation. The EC_{50} and E_{max} were estimated for individual concentration–response curves expressed as percentages of the contraction elicited by a high-K^+ solution by use of nonlinear least-squares regression (Graph Pad Prism 3.0, Graph Pad Software,

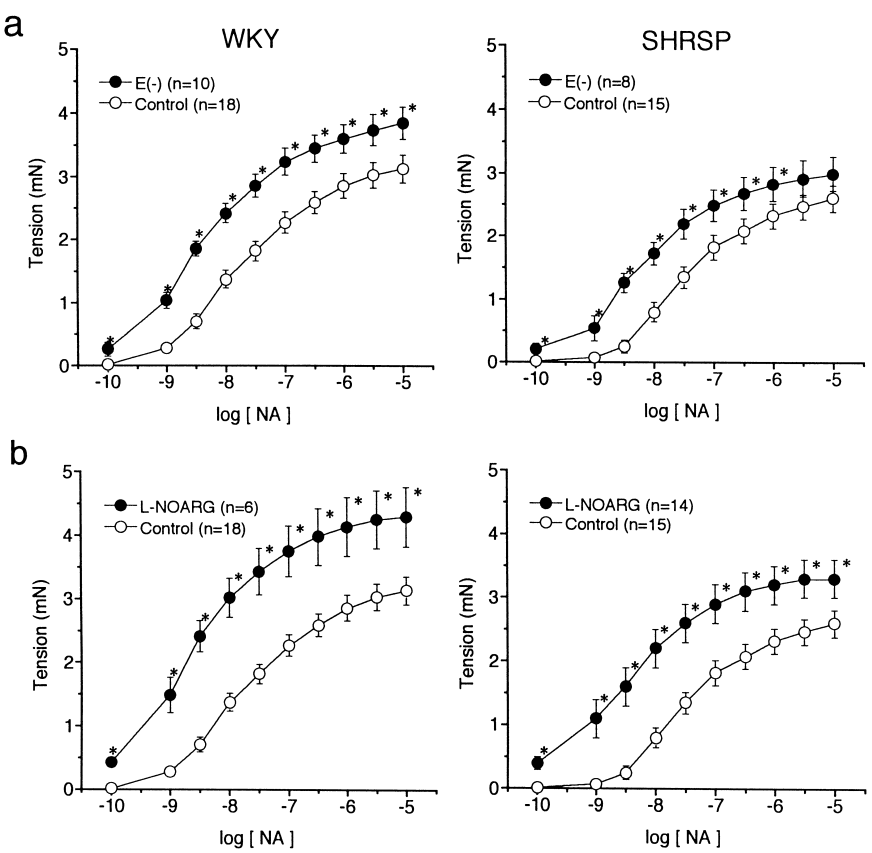

Fig. 2. Effects of endothelium removal or L-NOARG on noradrenaline-induced contraction in preparations from WKY (left) and SHRSP (right). The experiments were performed in the presence of propranolol 10^{-6} M. (a) Concentration–response curves measured in preparations with and without endothelium. Control and $E(-)$ indicate the curves obtained in the preparations with and without endothelium, respectively. (b) Concentration–response curves in the absence and presence of L-NOARG in endothelium-intact preparations. Control and L-NOARG represent the curves obtained in the absence and in the presence of L-NOARG, respectively. L-NOARG 10^{-4} M was applied 60 min prior to the concentration–response experiments. Other details are the same as those in Fig. 1.

USA). Differences in the results were analyzed by Student's *t*-test and P values less than 0.05 were considered significant.

3. Results

3.1. Body weight and blood pressure

The WKY and SHRSP weighed 341.4 ± 1.8 g ($n = 27$) and 263 ± 0.8 g ($n = 37$), respectively. The difference in body weight between WKY and SHRSP was significant ($P < 0.001$). The systolic blood pressure of the rats at the age of 16 weeks was 132.8 ± 0.7 mm Hg ($n = 27$) and 249.8 ± 0.5 mm Hg ($n = 37$) in WKY and SHRSP, respectively. The systolic blood pressure of SHRSP was significantly higher than that of WKY ($P < 0.001$).

3.2. Responses to noradrenaline

Noradrenaline up to 10^{-6} M caused concentration-dependent contraction in the pulmonary arterial rings from both WKY and SHRSP (Fig. 1). Concentrations of noradrenaline higher than 10^{-6} M caused tension decreases rather than increases in the preparations from both WKY and SHRSP. In the presence of the β-adrenoceptor antagonist propranolol (10^{-6} M), the tension decreases caused by higher concentrations of noradrenaline disappeared, and the maximum tension development by noradrenaline increased up to 10^{-5} M. Therefore, experiments with noradrenaline were performed in the presence of 10^{-6} M propranolol.

The removal of the endothelium caused a significant shift of the concentration–response curves to the left and upward in the preparations from WKY and SHRSP (Fig. 2a). After removal of the endothelium, the EC_{50} values for noradrenaline-induced contraction were significantly decreased in the preparations from WKY (Control; $1.47 \pm 0.12 \times 10^{-8}$ M ($n = 18$), $E(-)$; $4.41 \pm 1.2 \times 10^{-9}$ M ($n = 10$), $P < 0.001$) and SHRSP (Control; $2.23 \pm 1.2 \times 10^{-8}$ M ($n = 12$), $E(-)$; $6.59 \pm 1.3 \times 10^{-9}$ M ($n = 8$), $P < 0.001$). The EC_{50} values were comparable between the two strains with or without the endothelium. The E_{max} values obtained without the endothelium were significantly higher than those obtained with the endothelium in WKY (Control; $90.6 \pm 1.8\%$ ($n = 18$), $E(-)$; $107.4 \pm 2.5\%$ (n

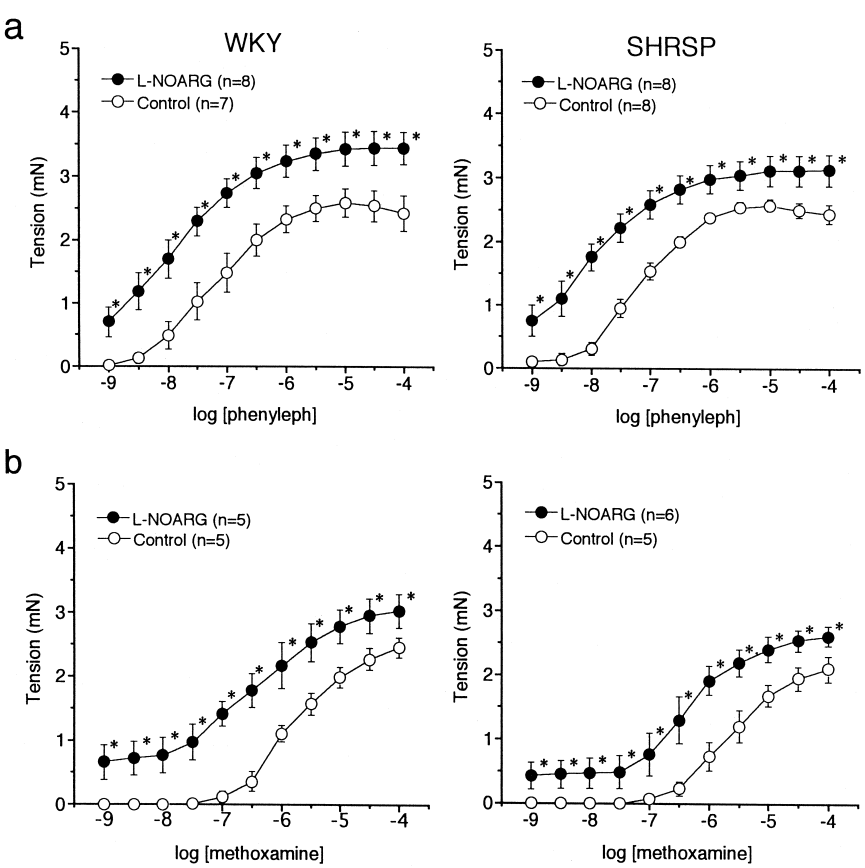

Fig. 3. Effects of L-NOARG on the contraction elicited by α_1-adrenoceptor agonists (phenylephrine and methoxamine). (a) Concentration–response curves for phenylephrine-induced contraction. (b) Concentration–response curves for methoxamine-induced contraction. L-NOARG at 10^{-4} M was applied 60 min prior to the experiments. Other details are the same as those in Figs. 1 and 2.

$= 10$), $P < 0.001$) and SHRSP (Control; $87.0 \pm 2.6\%$ ($n = 12$), $E(-)$; $96.4 \pm 3.9\%$ ($n = 8$), $P < 0.001$), respectively, although there was no significant difference between the two strains with or without the endothelium.

In endothelium-intact preparations, pretreatment with the NO synthase inhibitor L-NOARG 10^{-4} M caused a shift of the noradrenaline concentration–response curves to the left and upward (Fig. 2b). The contraction was changed almost independently of the concentration of noradrenaline. L-NOARG decreased. The EC_{50} values by 2- and 1.9-fold and increased E_{max} by about 25% and 18% in preparations from WKY (EC_{50}; $2.99 \pm 1.1 \times 10^{-9}$ M ($n = 6$), E_{max}; $113.0 \pm 2.8\%$ ($n = 6$)) and SHRSP (EC_{50}; $4.32 \pm 1.4 \times 10^{-9}$ M ($n = 10$), E_{max}; $102.2 \pm 2.2\%$ ($n = 10$)), respectively as compared to the values measured in the absence of L-NOARG. The EC_{50} values in the presence of L-NOARG were not significantly different between the two strains and from the endothelium-denuded preparations of WKY and SHRSP, respectively. The E_{max} in the presence of L-NOARG in WKY and SHRSP was $113.0 \pm 2.8\%$ ($n = 6$) and $102.2 \pm 2.2\%$ ($n = 10$), respectively, showing no significant difference between WKY and SHRSP. Therefore, no significant difference was observed in the change of concentration–response curve induced by L-NOARG or endothelial removal between the preparations from WKY and SHRSP.

3.3. Responses to α_1-adrenoceptor agonists

The selective α_1-adrenoceptor agonist phenylephrine caused concentration-dependent contraction in the preparations from WKY and SHRSP (Fig. 3a). The EC_{50} and E_{max} values for the phenylephrine-induced contraction were not significantly different between the preparations from WKY (EC_{50}; $5.89 \pm 1.5 \times 10^{-8}$ M ($n = 7$), E_{max}; $85.5 \pm 3.9\%$ ($n = 7$)) and SHRSP (EC_{50}; $6.03 \pm 1.3 \times 10^{-8}$ M ($n = 8$), E_{max}; $80.4 \pm 3.8\%$ ($n = 8$)).

Pretreatment with L-NOARG caused a shift of the concentration–response curves to the left and upward in the preparations from both strains. Phenylephrine at concentrations lower than 10^{-9} M did not change L-NOARG-induced basal tension. The threshold concentration of phenylephrine for tension development and the concentration at which the maximum contraction was induced were not altered by L-NOARG. In the presence of L-NOARG, the EC_{50} values were significantly decreased in the preparations from WKY ($1.62 \pm 1.2 \times 10^{-8}$ M ($n = 8$), $P < 0.01$) and SHRSP ($1.51 \pm 1.3 \times 10^{-8}$ M ($n = 8$), $P < 0.01$), there being no significant difference between the two strains. The E_{max} values for phenylephrine with L-NOARG in the preparations from WKY and SHRSP were significantly increased to $108.0 \pm 3.9\%$ ($n = 8$) and $114.7 \pm 3.4\%$ ($n = 8$), respectively ($P < 0.001$). Similarly to the

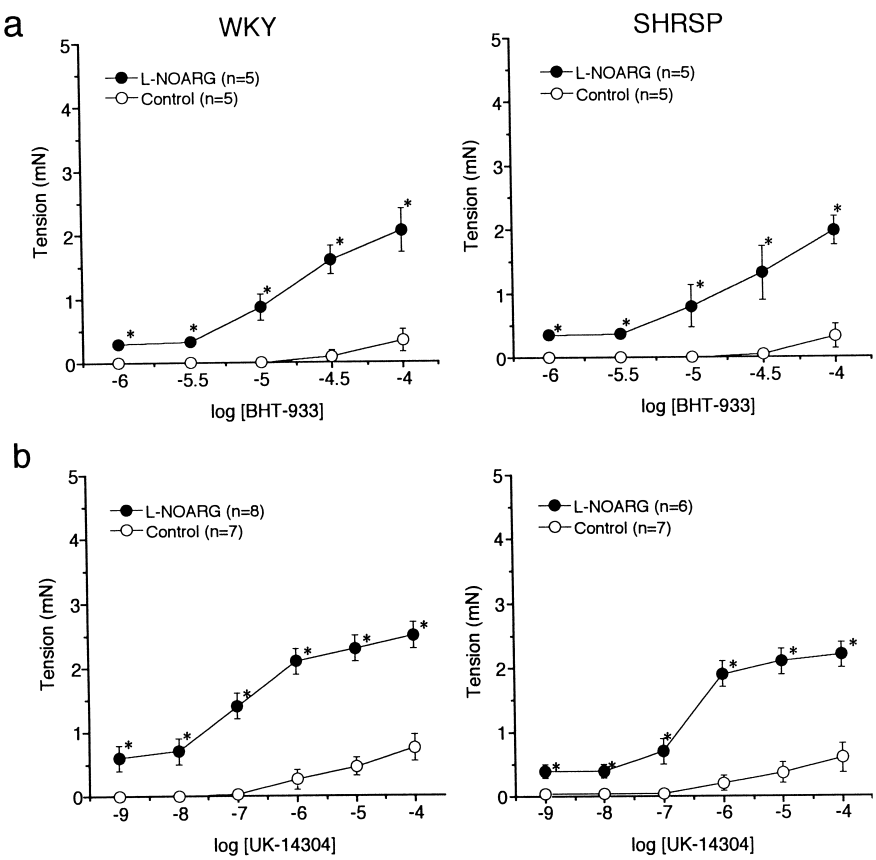

Fig. 4. Effects of L-NOARG on the contraction elicited by α_2-adrenoceptor agonists (BHT-933 and UK-14304). (a) Concentration–response curves for BHT-933-induced contraction. (b) Concentration–response curves for UK-14304-induced contraction. Other details are the same as those in Figs. 1 and 2.

noradrenaline curves, the change in the phenylephrine-induced contraction by L-NOARG was not dependent on the concentration of phenylephrine, and no difference was observed in the contractile response to phenylephrine between the preparations from WKY and SHRSP.

Similar results with L-NOARG were obtained with methoxamine, another selective α_1-adrenoceptor stimulant except that it had to be used in higher concentration to induce contraction (Fig. 3b). In the presence of L-NOARG, decreased EC_{50} values for methoxamine-induced contraction were observed in preparations from WKY (Control; $1.48 \pm 1.2 \times 10^{-6}$ M ($n = 5$), L-NOARG; $4.57 \pm 1.6 \times 10^{-7}$ M ($n = 5$), $P < 0.001$) and SHRSP (Control; $2.29 \pm 1.4 \times 10^{-6}$ M ($n = 5$), L-NOARG; $4.27 \pm 1.4 \times 10^{-7}$ M ($n = 6$), $P < 0.001$). The E_{max} values were significantly increased with L-NOARG in the preparations from WKY (Control; $85.9 \pm 3.3\%$ ($n = 5$), L-NOARG; $120.4 \pm 9.9\%$ ($n = 5$), $P < 0.001$) and SHRSP (Control; $79.3 \pm 7.5\%$ ($n = 5$), L-NOARG; $114.4 \pm 5.4\%$ ($n = 6$), $P < 0.001$). Removal of the endothelium resulted in a similar shift of the concentration–response curve for phenylephrine and methoxamine in the preparations from the two strains (data not shown).

3.4. Responses to α_2-adrenoceptor agonists

The selective α_2-adrenoceptor agonist BHT-933 induced a smaller contractile response at higher concentration than did the α_1-adrenoceptor agonists in the preparations both from WKY and SHRSP (Fig. 4a). The response to BHT-933 was markedly augmented in the presence of L-NOARG 10^{-4} M. In contrast to the responses to noradrenaline and α_1-adrenoceptor agonists, the augmentation by L-NOARG was dependent on the concentration of BHT-933 and increased as the concentration of the drug increased. In a similar manner, pretreatment with L-NOARG enhanced the concentration-dependent contractile response to another α_2-adrenoceptor agonist UK-14304 in the preparations from WKY and SHRSP (Fig. 4b).

The effects of L-NOARG on UK-14304-mediated responses in the preparations from WKY and SHRSP were dependent on the concentration of UK-14304. The maximum contraction was achieved at 10^{-4} M, and it was not

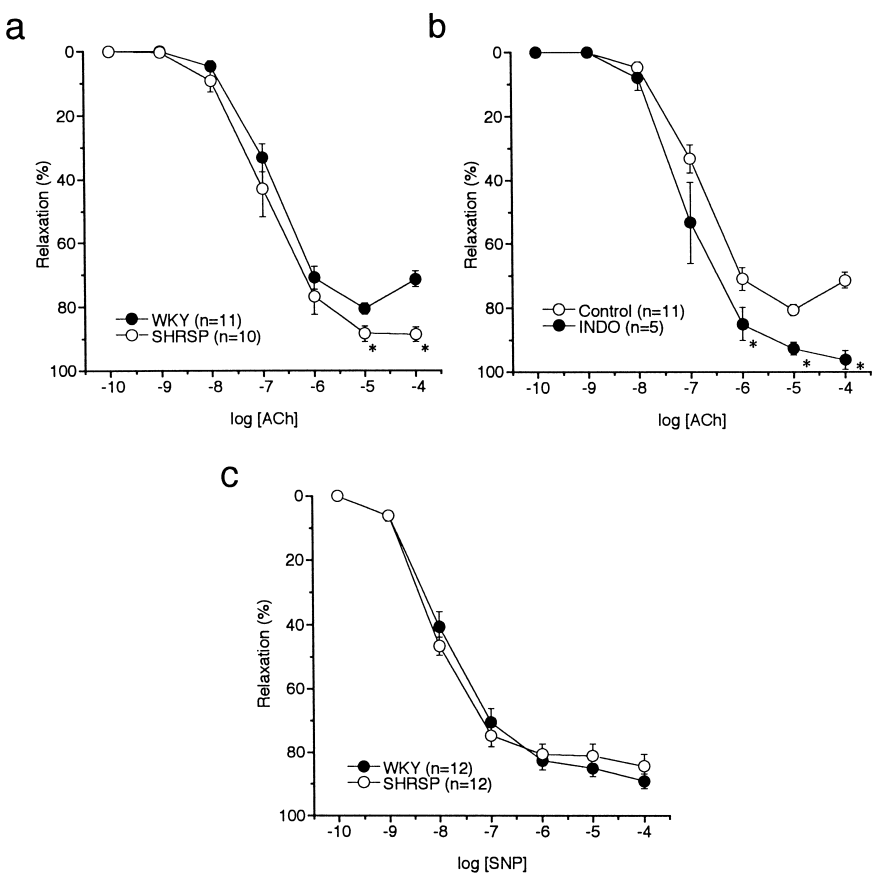

Fig. 5. Relaxation induced by acetylcholine (ACh) and sodium nitroprusside (SNP) in preparations of pulmonary arteries. (a) Concentration–response curves for ACh-induced relaxation in endothelium-intact preparations from WKY and SHRSP. (b) Effect of indomethacin (INDO) on the ACh-induced relaxation in the preparations from WKY. Indomethacin at 10^{-5} M was applied 30 min prior to the experiments. (c) Concentration–response curves for SNP-induced relaxation in endothelium-denuded preparations from WKY and SHRSP. Pulmonary arteries were contracted with 5×10^{-7} M norradrenaline and height of the contraction was taken as 100%. The relaxations are expressed as percentages of this height. Other details are the same as those in Fig. 1.

possible to determine EC_{50} and E_{max} values for preparations from WKY and SHRSP. The UK-14304-induced contraction was also augmented by removal of the endothelium. The maximum contractile responses to UK-14304 in the presence of L-NOARG (expressed as percentages of the high-K^+-induced contraction) did not differ from those observed after removal of the endothelium in preparations from WKY (L-NOARG; 88.1 ± 7.0% ($n = 8$), $E(-)$; 75.3 ± 3.7% ($n = 5$)) and SHRSP (L-NOARG; 88.8 ± 5.6% ($n = 6$), $E(-)$; 73.4 ± 3.9% ($n = 5$)).

3.5. Relaxation in response to acetylcholine

Acetylcholine, from 10^{-8} M to 10^{-4} M, produced concentration-dependent relaxation in the preparations contracted with noradrenaline 5×10^{-7} M (Fig. 5a). When high concentrations of acetylcholine were applied, the preparations from WKY showed a tendency to contract. This tendency disappeared completely in the presence of indomethacin (10^{-5} M; Fig. 5b). The relaxation was completely inhibited in the preparations from WKY and SHRSP by the removal of endothelium or pretreatment with L-NOARG (data not shown).

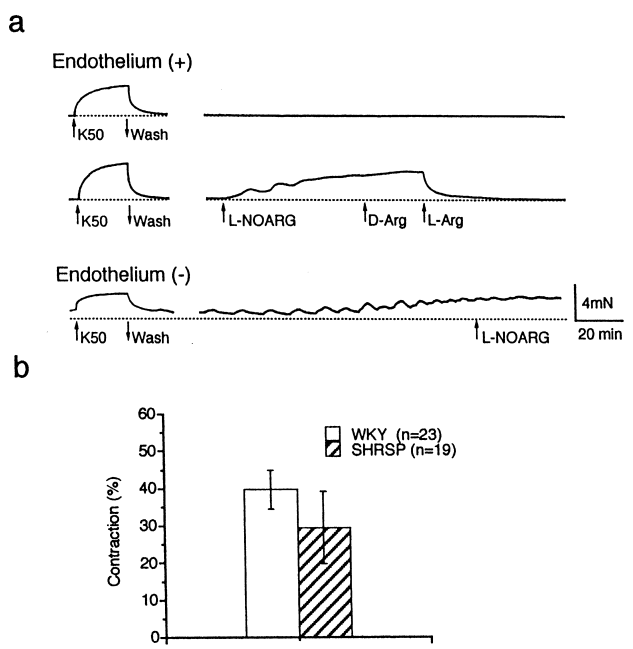

Fig. 6. Effects of L-NOARG on the pulmonary arteries from WKY and SHRSP. (a) Typical tracings of the effects of L-NOARG on the basal tension in endothelium-intact (+) and -denuded (−) preparations from WKY. K50, L-NOARG, D-Arg and L-Arg indicate the application of high-K^+ solution containing 50 mM K^+, N^G-nitro-L-arginine (10^{-4} M), D-arginine (10^{-3} M) and L-arginine (10^{-3} M), respectively. Dotted lines indicate the total relaxation elicited by application of verapamil (10^{-5} M) and papaverine (10^{-4} M) as described in Section 2. Note that L-NOARG was ineffective in the endothelium-denuded preparations. (b) Amplitude of L-NOARG-induced contraction in the endothelium-intact pulmonary arteries from WKY and SHRSP. The amplitude is expressed as a percentage of the high-K^+-induced contraction and shown as mean ± S.E. with the number of arteries in parentheses.

Sodium nitroprusside induced concentration-dependent relaxation in the preparations from WKY and SHRSP. The relaxation in response to sodium nitroprusside was not different in the preparations from WKY and SHRSP (Fig. 5c).

3.6. Effects of L-NOARG on the preparations under the resting state

Fig. 6 shows the effects of L-NOARG on endothelium-intact and -denuded pulmonary arteries. In the endothelium-intact preparations from WKY and SHRSP, the application of L-NOARG (10^{-4} M) caused a slow development of basal tension (contraction). The contraction was blocked by L-arginine (10^{-3} M) but not by D-arginine (10^{-3} M). There was no elevation of the basal tension (tone) in any of the endothelium-intact preparations from WKY or SHRSP unless L-NOARG was applied. When the endothelium was removed, 90% of the preparations (17 of 19 and 14 of 16 preparations from WKY and SHRSP, respectively) exhibited an elevation of the basal tension (tone) in the absence of L-NOARG, although the amplitude varied greatly. Moreover, L-NOARG (Fig. 6a) and L-arginine (data not shown) did not affect the tone of the endothelium-denuded preparations.

The amplitude of the L-NOARG-induced contraction was 39.7 ± 5.1% ($n = 23$) and 29.5 ± 9.6% ($n = 19$) of the maximum contractile response achieved by high K^+ solution in the preparations from WKY and SHRSP, respectively. There was no difference in the amplitude of L-NOARG-induced contractions in the preparations from WKY and SHRSP (Fig. 6b).

4. Discussion

The endothelium has been reported to depress the noradrenaline-induced contraction of arterial smooth muscle (Cocks and Angus, 1983; Carrier and White, 1985; Martin et al., 1986; Alosachie and Godfraind, 1988; Kaneko and Sunano, 1993; Matsuda et al., 1995). The results of the present study in pulmonary artery were in agreement with these reports. The noradrenaline-induced contraction was potentiated by endothelium removal in pulmonary artery and the potentiation was observed also following the application of L-NOARG. We considered that the inhibition of NO synthesis in the endothelium was responsible for the potentiation, as it is in other arteries (Vo et al., 1992; Adeagbo and Triggle, 1993; Kaneko and Sunano, 1993; Matsuda et al., 1995).

Noradrenaline has been shown to act on both α- and β-adrenoceptors (U'Prichard and Snyder, 1977). In the present study, the noradrenaline-induced contraction was reduced at high concentrations of the drug. The decrease disappeared following application of the β-adrenoceptor antagonist propranolol. Therefore, we considered that the

depression was mediated by stimulation of β-adrenoceptors, as reported in the pulmonary artery of Wistar rats (Priest et al., 1997). The present study was performed in the presence of propranolol to exclude the involvement of β-adrenoceptors when noradrenaline was used.

The change in noradrenaline-induced contraction by endothelium removal or by L-NOARG was not dependent on the concentration of noradrenaline. This suggested that the release of EDNO was not influenced by noradrenaline. Thus, we assumed that noradrenaline itself may not stimulate the release of EDNO but basally released EDNO may depress the contraction elicited by noradrenaline in rat pulmonary artery. Similar results and conclusions have been reported for the rat aorta (Martin et al., 1986). However, noradrenaline-induced EDNO release in rat mesenteric artery (White and Carrier, 1986) and aorta (Vinet et al., 1991; Vo et al., 1992; Adeagbo and Triggle, 1993; Kaneko and Sunano, 1993; Matsuda et al., 1995), which was observed in the absence of propranolol, is dependent on the noradrenaline concentration. This discrepancy may be due to tissue difference, but this remains to be determined.

Since the potentiation by L-NOARG or endothelial removal was not different between the preparations from WKY and SHRSP, it may be suggested that the release of EDNO by noradrenaline did not differ markedly between the preparations from WKY and SHRSP. We have reported previously that the inhibition of noradrenaline-induced contraction by EDNO was significantly reduced in aorta of SHRSP compared with that of WKY (Matsuda et al., 1995). This difference between pulmonary artery and aorta may be explained by difference in blood pressure in the arteries, as described below.

Depression of the α_1-adrenoceptor-induced contraction may be caused by basal release of EDNO, since the depression was not affected by the concentration of drug. This is in contrast to results reported previously for rat aorta (Carrier and White, 1985; Adeagbo and Triggle, 1993; Kaneko and Sunano, 1993; Matsuda et al., 1995), caudal artery (Vo et al., 1992) and mesenteric artery (White and Carrier, 1986), which indicated that NO was released from the endothelium in response to α_1-adrenoceptor stimulation. This difference might be explained by tissue differences.

We reported previously that the release of EDNO by α_1-adrenoceptor stimulation was reduced in the aorta from SHRSP (Matsuda et al., 1995). However, in the pulmonary artery, the basal release of EDNO, which depresses the α_1-adrenoceptor agonist-induced contraction, might be identical in both preparations from WKY and SHRSP, since there was no difference in the magnitude of the depression of the contraction.

The stimulation of α_2-adrenoceptors in the endothelium may cause the release of EDNO in the pulmonary artery, since L-NOARG potentiated the contraction in a manner dependent on the concentration of α_2-adrenoceptor agonist. Assuming that the basal release of EDNO is not influenced by α_2-adrenoceptor agonists and that a smaller contraction is more susceptible to EDNO than a larger contraction, the differences between L-NOARG and control in the concentration-dependence of the response to α_2-adrenoceptor agonists cannot be explained by the basal EDNO level.

The depression of the contraction in response to α_2-adrenoceptor stimulation by EDRF or EDNO has been reported in various vessels (Cocks and Angus, 1983; Egléme et al., 1984; White and Carrier, 1986; Kaneko and Sunano, 1993; Matsuda et al., 1995). It has also been reported that α_2-adrenoceptor stimulation causes endothelium-dependent relaxation in blood vessels (Cocks and Angus, 1983; Carrier and White, 1985; Miller and Vanhoutte, 1985; Angus et al., 1986; Kaneko and Sunano, 1993; Matsuda et al., 1995). Thus, it was suggested that the stimulation of α_2-adrenoceptors of the endothelium caused the release of EDNO, which in turn depressed the contraction. However, the present results for pulmonary artery may indicate that α_2-adrenoceptors may not play a major role in the noradrenaline-induced contraction, since the effect of L-NOARG on the response to noradrenaline was similar to that to the α_1-adrenoceptor agonist. It might be assumed that the contraction elicited by noradrenaline would be mainly due to α_1-adrenoceptor stimulation in smooth muscle and that the stimulation of endothelial α_2-adrenoceptor plays a minor role in depression of the contraction. It may imply that the affinity of noradrenaline for α_1-adrenoceptors or populations of α_1-adrenoceptors is greater than that for α_2-adrenoceptors in rat pulmonary artery; however, at present no information is available about this.

The depression of α_2-adrenoceptor agonist-induced contraction by EDNO was not different in pulmonary arteries from WKY and SHRSP, indicating that the release of EDNO elicited by α_2-adrenoceptor stimulation is identical in the preparations from WKY and SHRSP. This suggested that the function of the endothelium is not impaired in the pulmonary artery of SHRSP. This is also in contrast to our previous results, which indicated that the EDNO-induced relaxation in response to stimulation of α_2-adrenoceptors was markedly impaired in aorta of SHRSP (Sunano et al., 1996).

The endothelial function of the pulmonary arteries from WKY and SHRSP was studied by observing the relaxation elicited by acetylcholine in noradrenaline-precontracted preparations. The relaxation response to acetylcholine in pulmonary artery was thought to be mediated mainly by EDNO, since it was completely blocked by removal of the endothelium or by the application of L-NOARG, as reported with L-NAME (Yaghi et al., 1997). It has been reported that the endothelium-dependent relaxation in systemic arteries from SHR and SHRSP is impaired (Lüscher and Vanhoutte., 1988; Sunano et al., 1989; Watt and Thurston, 1989; Sunano et al., 1996). However, the present

results demonstrated that the extent of relaxation in the pulmonary artery from SHRSP was greater than that in the pulmonary artery from WKY. An unaltered endothelium-dependent relaxation in hypertensive rats has also been reported in renal arteries (Lüscher et al., 1988). Since the relaxation in the preparation from WKY was improved by the application of indomethacin, the release of a product(s) of arachidonic acid metabolism via the cyclooxygenase pathway (Flower, 1974) appeared to interfere with the relaxation in response to acetylcholine in this preparation. The involvement of this factor(s) in other vessels of WKY may be small as compared with that in hypertensive rats (Lüscher and Vanhoutte, 1986; Watt and Thurston, 1989; Diederich et al., 1990; see also Vanhoutte and Boulanger, 1995). Thus, it should be emphasized that the present results for pulmonary artery preparations were in marked contrast to those reported for other arteries.

It was also suggested in the experiment with acetylcholine that the release of EDNO was not altered in preparations from SHRSP, since the relaxation in the presence of indomethacin was identical in preparations from WKY and SHRSP. This was in agreement with observations for some systemic arteries of hypertensive rats, although the change in the response to acetylcholine was in the opposite direction (Ito and Carretero, 1992; Sawada et al., 1994). The identical relaxation to sodium nitroprusside indicated that the formation of cyclic GMP in the smooth muscle was not altered in vessels from SHRSP (Rapoport and Murad, 1983).

The impairment of endothelium-dependent relaxation in systemic arteries from hypertensive rats can be restored by chronic antihypertensive treatment (Shimamura et al., 1991; Sunano et al., 1992; Dohi et al., 1994; Takase et al., 1996). This indicates that the impairment is a secondary change due to sustained hypertension. The unaltered relaxation in the pulmonary artery preparation from SHRSP may be explained by the lower blood pressure in the artery, as reported by Aharinejad et al. (1996). Thus, this would be the main cause of the unaltered or rather increased EDNO-mediated relaxation and unaltered modulation of α-adrenoceptor agonist-induced contraction in the pulmonary artery from SHRSP despite the high blood pressure in systemic arteries.

The elevation of basal tension by L-NOARG can be explained by specific inhibition of the basal release of EDNO, since the elevation was counteracted by L-arginine but not by D-arginine. The observation that removal of the endothelium abolished the contractile effect of L-NOARG also supported this assumption. Thus, there is a basal release of EDNO in the pulmonary artery. This basal release of EDNO is not altered in preparations from SHRSP, since the effects of L-NOARG were similar in the endothelium-intact preparations from WKY and SHRSP.

In conclusion, our results suggested that the depression of contraction in response to α_1-adrenoceptor agonist by endothelium in pulmonary artery was brought about by basally released EDNO, while the depression of α_2-adrenoceptor agonist-induced contraction was mediated by EDNO released in response to receptor stimulation. The unaltered endothelium-dependent depression of contraction in the artery of SHRSP agreed with the observation that the endothelium-dependent relaxation in response to acetylcholine was not impaired in the preparation. The intact depression of α-adrenoceptor agonist-induced contraction and endothelium-dependent relaxation may be explained by the lower blood pressure in the pulmonary artery of SHRSP.

References

Adeagbo, A.S.O., Triggle, C.R., 1993. Interactions of nitric oxide synthase inhibitors and dexamethasone with α-adrenoceptor-mediated responses in rat aorta. Br. J. Pharmacol. 109, 495–501.

Aharinejad, S., Schraufnagel, D.E., Böck, P., MacKay, C.A., Larson, E.K., Miksovsky, A., Marks, S.C. Jr., 1996. Spontaneously hypertensive rats develop pulmonary hypertension and hypertrophy of pulmonary venous sphincters. Am. J. Pathol. 148, 281–290.

Alosachie, I., Godfraind, T., 1988. The modulatory role of vascular endothelium in the interaction of agonists and antagonists with α-adrenoceptors in the rat aorta. Br. J. Pharmacol. 95, 619–629.

Angus, J.A., Cocks, T.M., Satoh, K., 1986. α_2-Adrenoceptors and endothelium-dependent relaxation in canine large arteries. Br. J. Pharmacol. 88, 767–777.

Carrier, G.O., White, R.E., 1985. Enhancement of alpha-1 and alpha-2 adrenergic agonist-induced vasoconstriction by removal of endothelium in rat aorta. J. Pharmacol. Exp. Ther. 232, 682–687.

Clozel, M., Kuhn, H., Hefti, F., 1990. Effects of angiotensin converting enzyme inhibitors and of hydralazine on endothelial function in hypertensive rats. Hypertension 16, 532–540.

Cocks, T.M., Angus, J.A., 1983. Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. Nature 305, 627–630.

Cohen, R.A., Vanhoutte, P.M., 1995. Endothelium-dependent hyperpolarization beyond nitric oxide and cyclic GMP. Circulation 92, 3337–3349.

Diederich, D., Yang, Z., Bühler, F.R., Lüscher, T.F., 1990. Impaired endothelium-dependent relaxations in hypertensive resistance arteries involve cyclooxygenase pathway. Am. J. Physiol. 258, H445–H451.

Dohi, Y., Criscione, L., Pfeiffer, K., Lüscher, T.F., 1994. Angiotensin blockade or calcium antagonists improve endothelial dysfunction in hypertension: studies in perfused mesenteric resistance arteries. J. Cardiovasc. Pharmacol. 24, 372–379.

Egléme, C., Godfraind, T., Miller, R.C., 1984. Enhanced responsiveness of rat isolated aorta to clonidine after removal of the endothelial cells. Br. J. Pharmacol. 81, 16–18.

Flower, R.J., 1974. Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev. 26, 33–67.

Furchgott, R.F., 1984. The role of endothelium in the responses of vascular smooth muscle to drugs. Annu. Rev. Pharmacol. Toxicol. 24, 175–197.

Ishii, K., Chang, B., Kerwin, J.F. Jr., Huang, Z.J., Murad, F., 1990. N^{ω}-nitro-L-arginine: a potent inhibitor of endothelium-derived relaxing factor formation. Eur. J. Pharmacol. 176, 219–223.

Ito, S., Carretero, O.A., 1992. Impaired response to acetylcholine despite intact endothelium-derived relaxing factor/nitric oxide in isolated microperfused afferent arterioles of the spontaneously hypertensive rat. J. Cardiovasc. Pharmacol. 20 (Suppl. 12), S187–S189.

Kaneko, K., Sunano, S., 1993. Involvement of α-adrenoceptors in the endothelium-dependent depression of noradrenaline-induced contraction in rat aorta. Eur. J. Pharmacol. 240, 195–200.

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

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., Vanhoutte, P.M., 1988. Hypertension and endothelium-dependent responses. In: Vanhoutte, P.M. (Ed.), Vascular Smooth muscle, Peptides, Autonomic Nerves, and Endothelium. Raven Press, New York, NY, pp. 523–529.

Marletta, M.A., 1993. Nitric oxide synthase structure and mechanism. J. Biol. Chem. 268, 12231–12234.

Martin, W., Furchgott, R.F., Villani, G.M., Jothianandan, D., 1986. Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor. J. Pharmacol. Exp. Ther. 237, 529–538.

Matsuda, K., Sekiguchi, F., Tojo, M., Shimamura, K., Sunano, S., 1995. Effect of N^G-nitro-L-arginine on α-agonists-induced contraction of aortae from Wistar Kyoto rats and stroke-prone spontaneously hypertensive rats. J. Smooth Muscle Res. 31, 51–60.

Miller, V.M., Vanhoutte, P.M., 1985. Endothelial α_2-adrenoceptors in canine pulmonary and systemic blood vessels. Eur. J. Pharmacol. 118, 123–129.

Moore, P.K., al-Swayeh, 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 vasodilation in vitro. Br. J. Pharmacol. 99, 408–412.

Okamoto, K., Yamori, Y., Nagaoka, A., 1974. Establishment of the stroke-prone spontaneously hypertensive rat (SHR). Circ. Res. 34–35 (Suppl), 143–153.

Palmer, R.M.J., Rees, D.D., Ashton, D.S., Moncada, S., 1988. L-Arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biochem. Biophys. Res. Commun. 153, 1251–1256.

Priest, R.M., Hucks, D., Ward, J.P.T., 1997. Noradrenaline, β-adrenoceptor mediated vasorelaxation and nitric oxide in large and small pulmonary arteries of the rat. Br. J. Pharmacol. 122, 1375–1384.

Rapoport, R.M., Murad, F., 1983. Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. Circ. Res. 52, 352–357.

Rees, D.D., Palmer, R.M.J., Schulz, P.R., Hodson, H.F., Moncada, S., 1990. Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br. J. Pharmacol. 101, 746–752.

Sawada, Y., Sakamaki, T., Nakamura, T., Sato, K., Ono, Z., Murata, K., 1994. Release of nitric oxide in response to acetylcholine is unaltered in spontaneously hypertensive rats. J. Hypertens. 12, 745–750.

Shimamura, K., Osugi, S., Moriyama, K., Sunano, S., 1991. Impairment and protection of endothelium-dependent relaxation in aortae of various strains of spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 17 (Suppl. 3), S133–S136.

Sunano, S., Osugi, S., Kaneko, K., Yamamoto, K., Shimamura, K., 1992. Effects of chronic treatment with SQ29852 on spontaneous smooth muscle tone and endothelium-dependent relaxation in aorta of stroke-prone spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 19, 602–609.

Sunano, S., Osugi, S., Shimamura, K., 1989. Blood pressure and impairment of endothelium-dependent relaxation in spontaneously hypertensive rats. Experientia 45, 705–708.

Sunano, S., Zou, L.-B., Matsuda, K., Sekiguchi, F., Watanabe, H., Shimamura, K., 1996. Endothelium-dependent relaxation by α_2-adrenoceptor agonists in spontaneously hypertensive rat aorta. J. Cardiovasc. Pharmacol. 27, 733–739.

Takase, H., Moreau, P., Küng, C.F., Nava, E., Lüscher, T.F., 1996. Antihypertensive therapy prevents endothelial dysfunction in chronic nitric oxide deficiency. Hypertension 27, 25–31.

U'Prichard, D.C., Snyder, S.H., 1977. Differential labelling of α- and β-noradrenergic receptors in calf cerebellum membranes with ^{3}H-adrenaline. Nature 270, 261.

Vanhoutte, P.M., 1989. Endothelium and control of vascular function state of the art lecture. Hypertension 13, 658–667.

Vanhoutte, P.M., Boulanger, C.M., 1995. Endothelium-dependent responses in hypertension. Hypertens. Res. 18, 87–98.

Vanhoutte, P.M., Rubanyi, G.M., Miller, V.M., Houston, D.S., 1986. Modulation of vascular smooth muscle contraction by the endothelium. Annu. Rev. Physiol. 48, 307–320.

Vinet, R., Brieva, C., Pinardi, G., Penna, M., 1991. Modulation of α-adrenergic-induced contractions by endothelium-derived relaxing factor in rat aorta. Gen. Pharmacol. 22, 137–142.

Vo, P.A., Reid, J.J., Rand, M.J., 1992. Attenuation of vasoconstriction by endogenous nitric oxide in rat caudal artery. Br. J. Pharmacol. 107, 1121–1128.

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

White, R.E., Carrier, G.O., 1986. α_1- And α_2-adrenoceptor agonist-induced contraction in rat mesenteric artery upon removal of endothelium. Eur. J. Pharmacol. 122, 349–352.

Yaghi, A., Paterson, N.A.M., McCormack, D.G., 1997. Differential effect of nitric oxide synthase inhibitors on acetylcholine-induced relaxation of rat pulmonary and celiac artery rings. Can. J. Physiol. Pharmacol. 75, 279–286.