Endothelium-Dependent Relaxation in Pulmonary Arteries of L-NAME-Treated Wistar and Stroke-Prone Spontaneously Hypertensive Rats

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Abstract

To evaluate whether the elevated blood pressure induced by chronic treatment with \( N^\omega \)-nitro-L-arginine methyl ester (L-NAME) contributes to an impairment of endothelium-dependent relaxation (EDR), the effects of chronic treatment of Wistar rats with L-NAME on systolic blood pressure, pulmonary arterial blood pressure and EDR of the pulmonary arteries were studied and compared with those of stroke-prone spontaneously hypertensive rats (SHRSP). While the systolic blood pressure (SBP) of Wistar rats was increased above that of controls by chronic treatment with L-NAME, it was still significantly lower than that of SHRSP. Chronic treatment with L-NAME did not affect pulmonary arterial blood pressure. On the other hand, the pulmonary arterial blood pressure of SHRSP was slightly but significantly higher than that of the control normotensive Wistar Kyoto rats (WKY). EDR in response to acetylcholine in the pulmonary artery of L-NAME-treated rats was significantly smaller than that in control Wistar rats. The EDR markedly increased in the presence of L-arginine and completely disappeared in the presence of \( N^\omega \)-nitro-L-arginine. Indomethacin hardly affected EDR. In preparations from SHRSP, the EDR was not different from that in those from WKY. Relaxation induced by sodium nitroprusside was identical in all preparations. Elevation of SBP and the impairment of EDR observed in L-NAME-treated rats recovered two weeks following cessation of treatment. These results suggest that the impaired EDR in the pulmonary artery of L-NAME-treated rats is not due to an L-NAME-induced increase in blood pressure but due to the inhibition of nitric oxide synthase by the drug remaining in the endothelium.

Key words: chronic L-NAME treatment, endothelium-dependent relaxation (EDR), pulmonary artery, pulmonary arterial blood pressure, stroke-prone spontaneously hypertensive rats (SHRSP)

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Introduction

Endothelium-derived nitric oxide (EDNO) plays an important role in the regulation of contraction and tone of vascular smooth muscle (Lüscher and Vanhoutte, 1990; Moncada et al., 1991; Shimokawa and Takeshita, 1995). EDNO is synthesized from L-arginine by nitric oxide synthase (NOS) in the endothelium and activates soluble guanylyl cyclase in vascular smooth muscle. As a consequence, the level of guanosine 3':5'-cyclic monophosphate (cyclic GMP) in smooth muscle cells increases, leading to smooth muscle relaxation (Rapoport and Murad, 1983; Ignarro, 1989; Moncada et al., 1991). The synthesis of EDNO can be blocked by L-arginine analogues such as \(\text{N}^\omega\)-nitro-L-arginine (L-NOARG), \(\text{N}^\omega\)-monomethyl-L-arginine or \(\text{N}^\omega\)-nitro-L-arginine methyl ester (L-NAME) (Palmer et al., 1988; Moore et al., 1990; Rees et al., 1990), and the acute administration of these inhibitors of NOS to animals causes elevation of blood pressure (Aisaka et al., 1989; Gardiner et al., 1990a and 1990b; Moncada et al., 1991).

Chronic inhibition of NOS by L-NAME has also been reported to induce sustained hypertension in rats (Ribeiro et al., 1992; Bryant et al., 1995; Küng et al., 1995; Dowell et al., 1996; Takase et al., 1996; Henrion et al., 1997; Ledingham and Laverty, 1997; Moreau et al., 1998; Zhao et al., 1999; Sekiguchi et al., 2001). It has also been reported that endothelium-dependent relaxation (EDR) (Bryant et al., 1995; Küng et al., 1995; Takase et al., 1996; Zhao et al., 1999; Sekiguchi et al., 2001) and cyclic GMP production (Arnal et al., 1992) were decreased by this treatment, indicating some impairment of EDNO production. This may be the cause of hypertension in rats treated with L-NAME chronically. In these experiments, systemic arteries such as the aorta (Bryant et al., 1995; Zhao et al., 1999), carotid (Sekiguchi et al., 2001), iliac (Sekiguchi et al., 2001) and mesenteric arteries (Dowell et al., 1996; Takase et al., 1996; Zhao et al., 1999) were used to investigate the effects of chronic treatment with L-NAME on EDR. These arteries are thought to be directly affected by increased blood pressure. We reported previously that high blood pressure itself can be a cause of impairment of EDR in the aorta of stroke-prone spontaneously hypertensive rats (SHRSP) (Sunano et al., 1992 and 1993; Shimamura et al., 1998). Therefore, it is possible that the hypertension induced by chronic treatment with L-NAME itself is the cause of the impairment of EDR in these blood vessels.

As opposed to these blood vessels, the pulmonary artery is expected to be less affected by high blood pressure (hypertension), since the blood pressure in this vessel is known to be much lower than the blood pressure of systemic arteries (Aharinejad et al., 1996). It is also expected that the blood pressure of the pulmonary artery is less affected by chronic treatment with L-NAME than systemic arteries; at least the elevated level caused by the treatment is expected to be much lower than in the systemic arteries of hypertensive animals such as spontaneously hypertensive rats (SHR). Thus, it is of interest to study the effects of chronic treatment with L-NAME on pulmonary arterial pressure and on EDR of this vessel in normotensive rats.

In the present experiments, the effects of chronic treatment with L-NAME on systolic blood pressure, pulmonary blood pressure and EDR of the pulmonary artery were studied and compared with those of SHRSP.
Methods

Animals

Animals were handled according to “Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences” of the Physiological Society of Japan.

Male Wistar rats, Wistar Kyoto rats (WKY) and SHRSP of 5 weeks of age (Shimizu Laboratory Supplies Co. Ltd., Kyoto, Japan) were maintained in our animal facility at 22°C and 60% humidity under a 12 h light-dark cycle. Animals were given free access to normal chow (SP, Funabashi, Japan) and drinking water.

Wistar rats were assigned to three groups at 14 weeks of age as follows: 1) L-NAME-treated group (L-NAME-treated rats). Nine rats were given L-NAME (50 mg/100 ml) in the drinking water for 2 weeks (from 14 to 16 weeks of age). The daily intake of L-NAME was calculated as 64.8 ± 1.89 mg kg⁻¹ day⁻¹. 2) L-NAME treatment-stopped group (L-NAME-stopped rats). Nine rats were given L-NAME (50 mg/100 ml) in the drinking water for 2 weeks (from 14 to 16 weeks of age), and then given water without the drug for 2 weeks (from 16 to 18 weeks of age). 3) Control group (control rats). Five rats were given normal drinking water until sacrifice (18 weeks old).

Male SHRSP were also used to compare the effects of hypertension, with male normotensive WKY used as controls. These were maintained in our animal facility without the drug until sacrifice (18 weeks old).

Measurement of blood pressure

Systolic blood pressure (SBP) of the three groups of Wistar rats was measured three times a week using the tail-cuff method. Prior to the SBP measurement, the rats were warmed at 40°C for 10 min to obtain constant stable blood pressure values. The SBP of SHRSP and WKY was measured using the same method before being used for experiments.

Blood pressure in the pulmonary artery was measured by catheterization in closed-chest rats according to a previously reported method (Hayes et al., 1978; Stinger et al., 1981). Briefly, rats were anesthetized with α-chloralose and urethane (5 ml kg⁻¹ i.p. of a solution including 1% α-chloralose and 10% urethane). The right jugular vein was isolated and a cannula-catheter filled with heparinized saline was introduced into this vessel. After tying loosely, the catheter was inserted into the right atrium via the superior vena cava, and blood pressure changes monitored with a pressure transducer (Nihon-Kohden, Tokyo, Japan). The catheter was inserted approximately 1.5 cm further with careful rotation to ensure entry into the pulmonary artery. Completion of this insertion was ascertained by the drop in the pressure curve as reported previously (Hayes et al., 1978; Stinger et al., 1981). The catheter was then fixed in place by tightening the knot described above.

Preparations

Rats were killed by exsanguination from the vena cava under anesthesia with CO₂ at the age of 16 weeks (L-NAME-treated rats, non-treated SHRSP and WKY) or 18 weeks (L-NAME-stopped rats and control rats). The heart and lungs were removed en bloc, and placed in a modified
Tyrode’s solution (see composition below) while connective tissue and fat was removed and the left extrapulmonary artery dissected free. Ring preparations 1 mm in width were then cut from the vessel. In some preparations, the endothelium was removed by rubbing the inner surface of the lumen with a thin soft rubber band. Ring preparations of the thoracic aorta 1 mm in width were cut in a similar manner.

Solutions

The composition of the modified Tyrode’s solution was as follows (mM): NaCl, 137; KCl, 5.4; CaCl₂, 2.0; MgCl₂, 1.0; NaHCO₃, 11.9; NaH₂PO₄, 0.4; glucose, 5.6. This solution was equilibrated with a gas mixture of 95% O₂ and 5% CO₂ at 37°C. The pH of the solution at 37°C under these conditions was 7.3. K+-Tyrode’s solution was made by replacing all NaCl in the modified Tyrode’s solution with KCl, while 50 mM K-containing Tyrode’s solution (K50) was made by mixing the modified Tyrode’s and K+-Tyrode’s solutions.

Measurement of tension

Two tungsten wires 30 µm in diameter were inserted into the lumen of the ring preparations, and one of the tungsten wires was tied to a plastic holder. The holder was immersed in a 10 ml organ bath filled with the modified Tyrode’s solution (37°C). The other tungsten wire was connected to a force-transducer (Shinkoh, Nagano, Japan). Tension changes were measured isometrically under a stretching tension of 8 mN.

Preparations were equilibrated in the organ bath for at least 60 min. Prior to the experiments, two successive K50-induced contractions of 20 min duration were initiated with an interval of 20 min. These procedures were required to obtain constant results during the following experiments.

Endothelium-dependent relaxation (EDR) was induced by applying acetylcholine (ACh) cumulatively to preparations pre-contracted with 5 × 10⁻⁷ M noradrenaline (NA). When NA was applied, calcium disodium ethylenediaminetetraacetate (26 µM) was added to the Tyrode’s solution to prevent oxidation of NA. The effects of L-NOARG (10⁻⁴ M), L-arginine (10⁻³ M) or indomethacin (10⁻⁵ M) on ACh-induced EDR were examined by adding these drugs 30 min prior to the application of NA. Concentration-response curves for sodium nitroprusside (SNP)-induced relaxation were plotted for the endothelium-denuded preparations pre-contracted with 5 × 10⁻⁷ M NA by applying SNP cumulatively. 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. Relaxation responses were expressed as percentages of the initial pre-contraction.

Drugs

The following drugs were used: noradrenaline (NA, (-)-arterenol bitartrate salt, Sigma, St. Louis, MO, USA), acetylcholine hydrochloride (ACh, Wako Chem., Osaka, Japan), calcium disodium ethylenediaminetetraacetate (Wako Chem.), N⁶-nitro-L-arginine methyl ester (L-NAME, Sigma), N⁶-nitro-L-arginine (L-NOARG, Sigma), L-arginine (Sigma), indomethacin (Wako Chem.), sodium nitroprusside (SNP, Sigma), verapamil hydrochloride (Wako Chem.),
papaverine hydrochloride (Wako Chem.), α-chloralose (Wako Chem.), urethane (Wako Chem.). All drugs were dissolved in distilled water except indomethacin, which was dissolved in distilled water containing Na₂CO₃ (10⁻² M) and sonicated before use.

Statistics
Results are expressed as the mean ± SEM. The differences in the values were analyzed by Student’s t-test or one-way analysis of variance (ANOVA) followed by Bonferroni/Dunn’s post hoc test. The differences in concentration-response curves were analyzed by two-way ANOVA. P values of less than 0.05 were considered to be significant. EC₅₀ values (concentration of agonist required to induce half the maximum response) were obtained from individual concentration-response curves by fitting the data to the logistic equation. The curve fitting was carried out by use of Prizm 3 software (GraphPad Software Inc. San Diego, CA, USA). Logarithmic values of EC₅₀ (pD₂) were used for the statistical analysis.

Results

Body weight and blood pressure

The body weight of Wistar rats decreased during the period of treatment with L-NAME (65.8 ± 1.98 mg kg⁻¹ day⁻¹) and after 2 weeks of continuous treatment this was markedly less than that of control rats (Table 1). The body weight of these rats recovered following cessation of treatment, and after 2 weeks was not significantly different from that of control rats.

The SBP of Wistar rats increased gradually with continuous treatment with L-NAME and reached a maximum after 2 weeks (Fig. 1). The SBP gradually recovered following cessation of treatment with L-NAME, and when measured after 2 weeks was not significantly different from that of control rats (Table 1).

The SBP of age-matched SHRSP was significantly higher than that of WKY or L-NAME-treated rats but was not significantly different between control Wistar rats and WKY (Table 1).

Endothelium-dependent relaxation in the pulmonary arteries from L-NAME-treated, L-NAME-stopped and control rats

Pulmonary arterial preparations were pre-contracted with NA (5 × 10⁻⁷ M) to observe ACh-induced endothelium-dependent relaxation. The pre-contraction was not significantly different between preparations from control, L-NAME-treated and L-NAME-stopped rats (Table 2).

ACh induced concentration-dependent relaxation (Fig. 2). No relaxation was observed in endothelium-denuded preparations (data not shown). In preparations from L-NAME-treated rats, ACh-induced EDR was markedly reduced relative to that in preparations from control rats. The maximal relaxation in L-NAME-treated rats was 40.9 ± 1.67% (n=12), compared with control rats at 72.6 ± 1.67% (n=7) (P<0.05). This difference disappeared after cessation of treatment for 2 weeks, when there was no significant difference in maximal relaxation between preparations from control and L-NAME-stopped rats (79.0 ± 2.59%; n=10).
Effects of L-NOARG, L-arginine and indomethacin on endothelium-dependent relaxation in the pulmonary arteries

In the presence of L-NOARG (10⁻⁴ M), the pre-contraction induced by NA (5 × 10⁻⁷ M) was greater in preparations from control rats and L-NAME-stopped rats than in those pre-contracted with NA alone (Table 2). This was not the case in those from L-NAME-stopped rats (Table 2). The ACh-induced relaxation of pulmonary arteries from all rats was completely abolished by addition of L-NOARG (10⁻⁴ M) to the incubation solution (Fig. 3). Under these conditions, a tendency towards contraction was observed at higher concentrations of ACh especially in the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Pulmonary arterial pressure (mm Hg)</th>
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<tbody>
<tr>
<td>Wistar rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control rats</td>
<td>409 ± 6.2 (n=5)</td>
<td>135 ± 2.8f (n=5)</td>
</tr>
<tr>
<td>L-NAME-treated rats</td>
<td>376 ± 10.2* (n=9)</td>
<td>174 ± 4.8* (n=9)</td>
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<tr>
<td>L-NAME-stopped rats</td>
<td>411 ± 7.2* (n=9)</td>
<td>146 ± 3.0* (n=9)</td>
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<tr>
<td>WKY</td>
<td>368 ± 6.1 (n=5)</td>
<td>138 ± 1.0* (n=8)</td>
</tr>
<tr>
<td>SHRP</td>
<td>254 ± 10.4*†, § (n=7)</td>
<td>261 ± 11.5*†, § (n=7)</td>
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</table>

Data are expressed as mean ± SEM. n, number of animals used in the present experiment; L-NAME, Nω-nitro-L-arginine methyl ester; WKY, Wistar Kyoto rats; SHRP, stroke-prone spontaneously hypertensive rats. *: P<0.05 vs. control rats, †: P<0.05 vs. L-NAME-treated rats, §: P<0.05 vs. WKY.
Table 2  Pre-contraction induced by noradrenaline (5 × 10⁻7 M)

<table>
<thead>
<tr>
<th></th>
<th>Noradrenaline pre-contraction (K50 contraction = 100%)</th>
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<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Wistar rats</td>
<td></td>
</tr>
<tr>
<td>control rats</td>
<td>97.2 ± 5.60 (n=7)</td>
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<tr>
<td>L-NAME-treated rats</td>
<td>111.5 ± 6.79 (n=12)</td>
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<tr>
<td>L-NAME-stopped rats</td>
<td>89.2 ± 3.51 (n=10)</td>
</tr>
<tr>
<td>WKY</td>
<td>81.7 ± 8.39 (n=10)</td>
</tr>
<tr>
<td>SHRSP</td>
<td>91.5 ± 2.21 (n=11)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. n, number of animals used in the present experiment; L-NAME, N⁰-nitro-L-arginine methyl ester; L-NOARG, N⁰-nitro-L-arginine; WKY, Wistar Kyoto rats; SHRSP, stroke-prone spontaneously hypertensive rats. *: P<0.05 vs. value of Control data.

Fig. 2. Concentration-response curves for acetylcholine (ACh)-induced endothelium-dependent relaxation in the pulmonary arteries from control, L-NAME-treated and L-NAME-stopped rats. Relaxation was induced by applying ACh cumulatively to preparations precontracted with 5 × 10⁻⁷ M noradrenaline. Asterisks and daggers indicate significant differences from the value of control rats and from that of L-NAME-treated rats, respectively (**: P<0.001, ††: P<0.001, two-way ANOVA).

preparations from L-NAME-treated rats.

In preparations from L-NAME-treated rats the ACh-induced relaxation was reduced, but recovered in the presence of L-arginine (10⁻³ M) (Fig. 3b) to a level comparable with that in control rats (c.f. Fig. 3a). Such potentiation of relaxation was not observed in preparations from control and L-NAME-stopped rats where the ACh-induced relaxation was not impaired. The pre-contraction induced by NA became significantly smaller in preparations from L-NAME-treated
Indomethacin (10⁻⁵ M) hardly restored the reduced ACh-induced relaxation in preparations from L-NAME-treated rats, although a slight increase in relaxation was observed at a high concentration of ACh (10⁻⁴ M) (Fig. 4). NA-induced pre-contraction in the presence of indomethacin was not significantly different between preparations from control and L-NAME-treated rats (P>0.05) with control rats at 77.0 ± 2.65% (n=6) and L-NAME-treated rats at 82.6 ± 5.45% (n=6) where the K50-induced contraction was 100%.

**Relaxation induced by sodium nitroprusside (SNP)**

SNP induced relaxation in endothelium-denuded preparations from all of the control, L-arginine, and L-NAME-treated rats. The relaxation response was significantly increased in the presence of L-arginine only in preparations from L-NAME-treated rats. **Fig. 3.** Effects of N⁶-nitro-L-arginine (L-NOARG) and L-arginine on the concentration-response curves for ACh-induced relaxation in the pulmonary arteries from control (a), L-NAME-treated (b) and L-NAME-stopped rats (c). Control, L-NOARG and L-arginine indicate in the absence of both L-NOARG and L-arginine, in the presence of L-NOARG (10⁻⁴ M) and in the presence of L-arginine (10⁻³ M), respectively. Asterisks indicate significant differences from the value of the Control of preparations from each group of rats (**: P<0.001, two-way ANOVA). Note the significant increase in the relaxation response in the presence of L-arginine only in preparations from L-NAME-treated rats.
NAME-treated and L-NAME-stopped rats pre-contracted with NA (5 × 10⁻⁷ M) (data not shown). The maximal relaxation was 97.5 ± 0.75% (n=6) in control rats, 97.2 ± 0.54% (n=6) in L-NAME-treated rats and 96.0 ± 0.54% (n=4) in L-NAME-stopped rats. The pD₂ values of the concentration-response curves for SNP of 7.88 ± 0.055 (n=6) for control rats, 7.93 ± 0.106 (n=6) for L-NAME-treated rats and 7.53 ± 0.193 (n=4) for L-NAME-stopped rats were not significantly different between these preparations.

Endothelium-dependent relaxation induced by ACh in the aortae and the pulmonary arteries from WKY and SHRSP

EDR in the aortic preparations from SHRSP was significantly reduced relative to that in preparations from WKY (Fig. 5a). On the other hand, no significant difference was observed between the EDR observed in pulmonary arterial preparations from WKY and SHRSP (Fig. 5b).

Pulmonary arterial blood pressure

Pulmonary arterial blood pressure was measured to determine whether chronic treatment with L-NAME induces pulmonary hypertension, and whether the blood pressure contributes to the impaired EDR in the pulmonary arterial preparations from L-NAME-treated rats.

The pulmonary arterial blood pressures measured by pulmonary arterial catheterization are shown in Table 1. The blood pressure in L-NAME-treated rats was similar to that of control rats, and significantly lower than that of SHRSP, which was slightly but significantly higher than that of WKY.
F. SEKIGUCHI et al.

Chronic administration of L-NAME caused an elevation of blood pressure in Wistar rats as previously reported (Arnal et al., 1992 and 1993; Baylis et al., 1992; Ribeiro et al., 1992; Bryant et al., 1995; Küng et al., 1995; Dowell et al., 1996; Takase et al., 1996; Henrion et al., 1997; Ledingham and Laverty, 1997; Moreau et al., 1998; Zhao et al., 1999; Sekiguchi et al., 2001). In the present study, we showed that while chronic treatment of Wistar rats with L-NAME elevated SBP, the pulmonary arterial blood pressure was unchanged. We have demonstrated that EDR of the pulmonary artery from these rats was markedly impaired. All of these changes recovered following cessation of treatment.

Impairment of the relaxation of blood vessels following chronic treatment with L-NAME has been reported in systemic arteries (Bryant et al., 1995; Küng et al., 1995; Takase et al., 1996; Zhao et al., 1999). L-NAME has been shown to inhibit the synthesis of NO in the endothelium (Rees et al., 1990; Pfeiffer et al., 1996), and such inhibition has been reported to enhance vascular smooth muscle contraction (Martin et al., 1986; Alosachie and Godfraind, 1988; Osugi et al., 1990; Kaneko and Sunano, 1993). We have also observed that EDR of the aorta and of the carotid and iliac arteries from L-NAME-treated rats was impaired (Sekiguchi et al., 2001). This impairment of EDR in arteries of L-NAME-treated rats is thought to be mainly due to inhibition of NO production by the drug (Arnal et al., 1992; Baylis et al., 1992; Ribeiro et al., 1992). On the other hand, Zhao et al. (1999) reported that the long term vascular effects of L-NAME are not solely mediated by inhibition of EDNO synthesis. In addition, it has been reported in SHR or SHRSP that high systemic blood pressure itself can impair endothelial function and thus EDR,
as these changes could be prevented or would recover by lowering the blood pressure in these animals (Clozel et al., 1990; Sunano et al., 1992 and 1993; Dohi et al., 1994; Shimamura et al., 1998). As mentioned above, treatment with L-NAME induces an elevation of blood pressure. These experiments investigating EDR in blood vessels from L-NAME-treated rats were performed in the absence of a NOS inhibitor. Nevertheless, EDR of the blood vessels was reported to be impaired. Thus, it is possible that elevated blood pressure contributes to the impairment of EDR in arteries of L-NAME-treated rats. In those experiments, systemic arteries, which are directly influenced by the elevation of blood pressure, were used to investigate the effects of chronic treatment with a NOS inhibitor on EDR, and therefore it is possible that sustained hypertension is involved in the impairment of EDR. In fact, as shown in the present study, treatment with L-NAME induced hypertension and impairment of EDR, both of which recovered with similar time courses following cessation of treatment. In addition, it has also been reported that EDR of the pulmonary artery of pulmonary hypertensive rats was impaired (Altiere et al., 1986; Ito et al., 1988; Nakazawa et al., 1999). Therefore, there might be some causal relationship between elevation of blood pressure and the impairment of EDR.

The pulmonary artery is a vessel with a much lower pressure than in systemic vessels (Aharinejad et al., 1996) as has been confirmed in the present study where pulmonary arterial pressure was shown to be markedly lower than the systemic arterial pressure measured by the tail-cuff method. In addition, we have also shown that chronic treatment with L-NAME did not affect the pulmonary arterial blood pressure. Nevertheless, EDR in the pulmonary artery of L-NAME-treated rats was markedly impaired. These results clearly indicate that change in blood pressure does not contribute to the impaired EDR in the pulmonary artery of L-NAME-treated rats.

In SHRSP, on the other hand, the pulmonary arterial blood pressure was significantly higher than that of WKY, although the blood pressure was still much lower than that in the respective systemic arteries. These results are consistent with the previous report in SHR (Aharinejad et al., 1996). In spite of higher blood pressure, the EDR in the pulmonary artery from SHRSP was not different from that in the preparations from WKY. This result is consistent with that reported by Matsuda et al. (2000). These results indicate that the pulmonary arterial blood pressure of SHRSP is still too low to impair the endothelium although the blood pressure was higher than that of WKY. This would indicate that the impaired EDR in the pulmonary artery of L-NAME-treated rats is not due to the elevation of blood pressure induced by the chronic treatment.

Since the relaxation of the pulmonary artery was completely abolished by the removal of the endothelium or by an application of L-NOARG, the relaxation is thought to be mediated by EDNO. The present in vitro experiments with the pulmonary artery from chronic L-NAME-treated rats were performed after soaking the preparations in normal Tyrode’s solution in the absence of L-NAME for a period of 3 to 4 hrs, during which the solution was changed at least 5 times. Thus, in preparations from L-NAME-treated rats, the augmentation of EDR by L-arginine (a substrate of NOS) is thought to be due to antagonism of the drug by residual L-NAME remaining in the endothelium. In support of this, Zhao et al. (1999) reported that L-NOARG (a metabolite of L-NAME) remained in the aorta isolated from L-NAME-treated rats for a long
They also observed that the concentration of L-arginine in the serum was not reduced by chronic L-NAME-treatment. Thus, it is likely that the impairment of EDR in the pulmonary artery from L-NAME-treated rats is caused by residual L-NAME or L-NOARG remaining in the endothelium.

The formation and action of cyclic GMP may not be altered by treatment with L-NAME, as the effect of SNP, which is known to be mediated by NO formation that activates soluble guanylyl cyclase and increases cyclic GMP level (Rapoport and Murad, 1983; Ignarro, 1989; Moncada et al., 1991), was not altered by this treatment. The involvement of (a) product(s) of the arachidonic acid cascade via the cyclooxygenase pathway in the impairment of relaxation has been reported in arteries of SHR (Diederich et al., 1990). However, this would not be the case in the pulmonary artery of L-NAME-treated rats, since the impaired relaxation was not improved to the level of control rats by addition of indomethacin, an inhibitor of cyclooxygenase (Mizuno et al., 1982; Needleman et al., 1986).

The observations that the elevated blood pressure of L-NAME-treated rats gradually fell after the cessation of treatment, and that it took more than 2 weeks to recover to the level of control rats, suggest the possibility that the clearance of the drug from endothelial cells is quite slow. It is also suggests that L-NAME does not remain in the preparations of L-NAME-stopped rats, since the EDR was identical with that in the preparations of control rats and not increased by application of L-arginine. However, we have not observed the time course of the change of EDR and the content of L-NAME in the tissue after cessation of treatment. Thus, further investigation is required to evaluate the clearance of L-NAME.

In conclusion, these results suggest that the impairment of relaxation in the pulmonary artery of L-NAME-treated rats is not due to a maintained hypertension but due to the inhibition of NO synthesis by the drug remaining in the endothelial cells.

References


L-NAME-treated rats pulmonary artery


Moore, P.K., al-Swayeh, O.A., Chong, N.W.S., Evans, R.A. and Gibson, A. (1990). L-N\textsuperscript{ω}-nitro arginine (L-


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