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Restriction of Nitric Oxide Rather than Elevated Blood Pressure is Responsible for Alterations of Vascular Responses in Nitric Oxide-Deficient Hypertension

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Summary

The responsiveness of isolated high-pressure (aorta, renal artery) and low-pressure vessels (pulmonary artery) was compared during systemic hypertension induced by chronic inhibition of nitric oxide synthesis by N^G-nitro-L-arginine methyl ester (L-NAME) in rats. L-NAME (40 mg/kg/day) was given to animals in their drinking water. After 4 weeks of L-NAME treatment, systolic blood pressure increased by 37 % as compared with that in the control group. Chronic L-NAME treatment resulted in significant reduction of endothelium-dependent relaxation to acetylcholine (10^{-8} to $3x10^{-6}$ mol/l) in both types of vessels. The reduced relaxation was not influenced by acute pretreatment with indomethacin (10^{-5} mol/l), however, it was further reduced by acute pretreatment with additional L-NAME (10^{-4} mol/l). L-arginine (10^{-4} mol/l) improved the reduced relaxation. Endothelium-independent relaxation to sodium nitroprusside (10^{-9} to 10^{-6} mol/l) was unaffected by L-NAME treatment. β-adrenoceptor-mediated relaxation to isoprenaline (10^{-8} to $3x10^{-6}$ mol/l) was also not influenced by chronic L-NAME treatment. Similar alterations in the responsiveness of high- and low-pressure vessels indicate rather the decisive role of nitric oxide restriction than that of elevated blood pressure in their development.

Key words

Nitric oxide – L-NAME – Hypertension – Pulmonary and systemic vascular reactivity – Rat

Introduction

The experimental hypertension induced by long-term inhibition of nitric oxide (NO) synthesis is characterized, besides a nonspecific pressure load on the vascular wall common to all hypertensions, by a specific restriction of NO levels. This fact by itself interferes with the modulatory capacity of blood vessels (Ignarro 1989).

The systemic (high-pressure) and pulmonary (low-pressure) vessels have different capacity for adaptation to these influences. Moreover, controversies still exist as to the contribution of NO to the control of pulmonary vascular tone. While some studies suggest that NO regulates pulmonary vascular responses to vasoactive agents as well as normoxic vasomotor tone (Ignarro *et al.* 1987, MacLean *et al.* 1993, Fineman *et al.* 1991), some others report no effect of NO on normoxic pulmonary tone (Nishiwaki *et al.* 1992, Hampl *et al.* 1993, Leeman *et al.* 1994).

The present experiments, therefore, compare the responsiveness of high-pressure (aorta, renal artery) and low-pressure vessels (pulmonary artery) during chronic hypertension induced by 4-week inhibition of NO synthesis in rats. Our primary objective was to determine the role of NO and/or elevated blood pressure in the modulation of vasomotor tone to different dilatory and constrictory stimuli.

Methods

Normotensive male Wistar rats (12–15 weeks of age) were offered to drink either water containing

N^G-nitro-L-arginine methyl ester (L-NAME, 40 mg/kg/day) to inhibit NO synthesis (experimental group, n=20), or tap water (control group, n=12). All animals were housed at a temperature of 22 °C in individual metabolic cages and fed a regular pellet diet. Systolic blood pressure was measured indirectly in pre-warmed rats by the tail-cuff method. After 4 weeks, systolic blood pressure in the experimental group was 160±3 mm Hg, i.e. higher by 37 % than in the control group (124±2 mm Hg). At that time, animals were sacrificed by decapitation and high-pressure (aorta, renal artery) and low-pressure vessel (pulmonary artery) were dissected free for acute experiments. The vessels were placed in an ice-cold Krebs solution, cleaned of connective tissue and cut into segments of about 4 mm long. The individual segments were attached between an isometric force transducer (Sanborn FT 10) and a holder under a tension of 40 mN for the aorta and 20 mN for the renal and pulmonary artery in a 20 ml organ bath containing Krebs solution as described earlier (Holécyová *et al.* 1993).

After a resting period of 60 min, the concentration-response curves to individual agonists were obtained. All relaxations were determined in the vessels precontracted with noradrenaline (NA, $3x10^{-7}$ mol/l). Some relaxations to acetylcholine (ACh) were induced in the presence of indomethacin (INDO, 10^{-5} mol/l) to prevent the production of vasoactive prostanoids, or in the presence of L-arginine (10^{-4} mol/l) to provide a substrate for NO synthase. In others, additional administration of L-NAME (10^{-4} mol/l) into the bath was used to obtain complete inhibition of NO synthesis.

All drugs (Sigma Chemical Co.) were dissolved in distilled water, with the exception of indomethacin, which was prepared in an equimolar concentration of sodium carbonate.

Statistical analysis

All relaxations were expressed as percentage of the initial precontraction to NA. All values are given as means ± S.E.M. Statistical evaluation of the data was performed by ANOVA with P values adjusted for multiple comparisons by Bonferroni. $P<0.05$ was considered to be significant.

Results

Effect of chronic L-NAME on relaxation responses

ACh (10^{-8} to $3x10^{-6}$ mol/l) elicited concentration-dependent relaxation of both high- (aorta, renal artery) and low-pressure (pulmonary artery) control vessels precontracted with $3x10^{-7}$ mol/l of NA. As shown in Figure 1, the maximal relaxation evoked by ACh ($3x10^{-6}$ mol/l) was 89.84±2.26 % in the aorta, 86.03±2.18 % in the renal artery and 79.78±1.60 % in the pulmonary artery. Chronic treatment with L-NAME (40 mg/kg/day) significantly reduced the relaxation in the aorta to 60.14±3.50 % in the renal artery to 61.71±5.34 % and only to 41.32±7.76 % in the pulmonary artery. L-arginine (10^{-4} mol/l) partially restored the reduced relaxation in renal and pulmonary arteries, but not in the aorta.

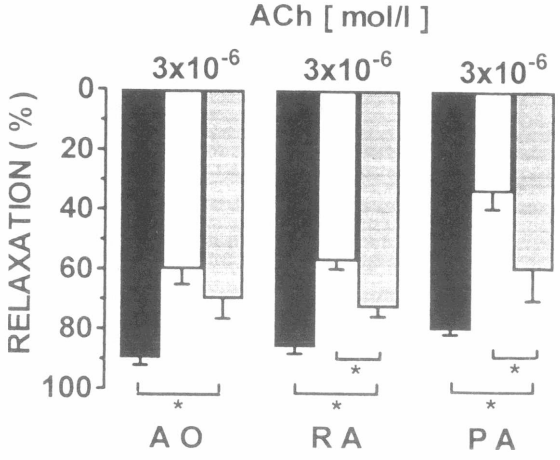

Fig. 1
*The mean maximum relaxation to acetylcholine in control vessels (full column), vessels chronically treated with L-NAME (open column) and after acute pretreatment with L-arginine (gray column). * $p<0.05$. AO – aorta, RA – renal artery, PA – pulmonary artery.*

In contrast to ACh, chronic treatment with L-NAME had no effect on relaxation induced by sodium nitroprusside (10^{-9} to 10^{-6} mol/l). Both types of vessels relaxed completely, indicating that the ability of vascular smooth muscles to relax was not changed (data not shown).

Table 1
The mean maximum relaxation to acetylcholine (%) in control and L-NAME treated animals after acute pretreatment with either INDO (10^{-5} mol/l) or L-NAME (10^{-4} mol/l)

Group	Control	Chronic L-NAME
Aorta		
Without pretreatment	91.07±3.60	40.56±3.68*
Pretreatment with INDO	97.35±3.01	61.72±4.57
Pretreatment with L-NAME	20.91±3.77	−6.31±10.10
Pulmonary Artery		
Without pretreatment	87.73±2.85	45.74±5.33*
Pretreatment with INDO	92.97±4.17	46.39±17.00
Pretreatment with L-NAME	1.74±3.82	2.93±1.13

** $p<0.05$ vs. control*

When acute pretreatment with indomethacin (10^{-5} mol/l) was used to block cyclooxygenase activity, neither the control, nor the residual relaxation to ACh in L-NAME treated vessels were influenced (Table 1).

Acute pretreatment with additional L-NAME (10^{-4} mol/l), however, reduced the maximum relaxation to ACh in control aortas while it reversed relaxation to contraction in aortas chronically treated with L-NAME. In the pulmonary arteries, acute pretreatment with L-NAME nearly completely abolished the control as well as the residual relaxation to ACh after chronic L-NAME treatment (Table 1).

β-adrenoceptor agonist isoprenaline (10^{-8} to $3x10^{-6}$ mol/l) induced concentration-dependent relaxation in both types of vessels. Maximal control relaxation, obtained in the aorta at the concentration $3x10^{-6}$ mol/l, was 89.09±1.31 %. In renal and pulmonary arteries, the maximal relaxation averaged 70.92±9.02 % and 71.33±4.02 %, respectively, and was reached at a concentration 10^{-6} mol/l. Chronic L-NAME did not influence the relaxation to isoprenaline. The mean maximum was 85.07±5.21 % in the aorta, 70.92±6.40 % in the renal artery and 74.85±3.69 % in the pulmonary artery.

Effect of chronic L-NAME on contraction to exogenous noradrenaline

NA (10^{-9} to 10^{-5} mol/l) induced concentration-dependent contraction in both types of vessels (Fig. 2). Maximal control contraction averaged 16.54±0.70 mN/mm^2 in the aorta, 12.04±0.77 mN/mm^2 in the renal artery and 13.26±0.80 mN/mm^2 in the pulmonary artery. Chronic L-NAME treatment increased the maximum contraction only in the renal (by 42 %) and pulmonary artery (by 36 %) as compared to the corresponding controls. However, the sensitivity to the agonist increased in all the vessels studied.

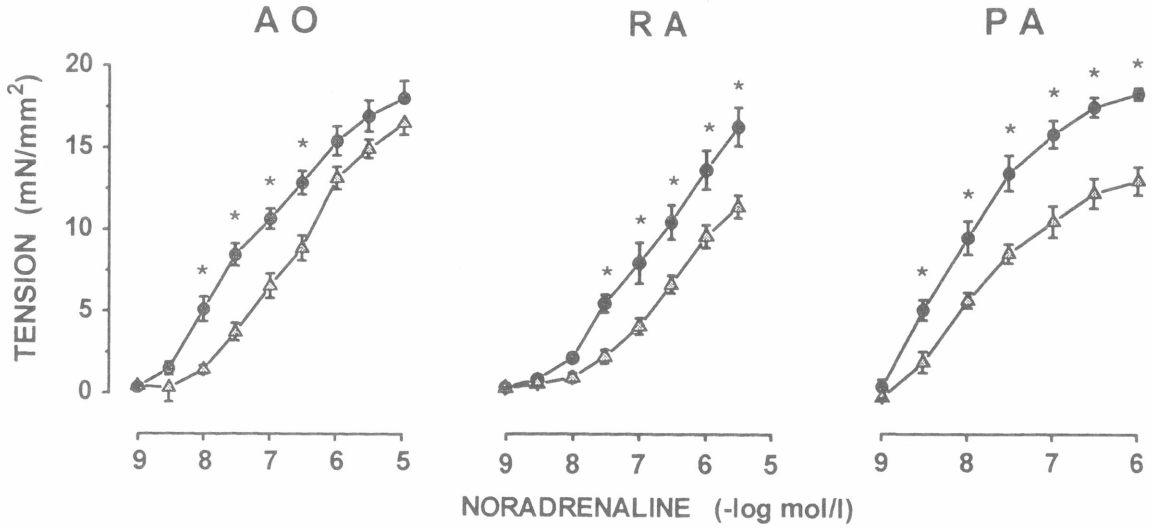

Fig. 2
*Concentration-dependent contraction to noradrenaline in rat aorta (AO), renal artery (RA) and pulmonary artery (PA). triangles – control vessels, dots – vessels chronically treated with L-NAME. Points represent the mean ± S.E.M. * $p<0.05$.*

Discussion

The present experiments have confirmed several previous studies that acute and chronic inhibition of NO synthesis by L-NAME induces a marked elevation of systemic blood pressure (Rees *et al.* 1990, Lahera *et al.* 1991, Deng *et al.* 1993, Zanchi *et al.* 1995). In spite of the elevation of systemic blood pressure, Hampl *et al.* (1993), in a model very close to ours, found no significant changes of pulmonary blood pressure in rats 3 weeks after L-NAME treatment. With regard to this observation we used the pulmonary artery as a representative of low-pressure vessels.

In our model, a depression of endothelium-dependent relaxation to ACh was found in both high- and low-pressure vessels. The results supplement those obtained after acute or chronic inhibition of NO synthesis by L-NAME in the rat mesenteric artery (Deng *et al.* 1993, Zanchi *et al.* 1995), the rabbit renal (Kitagawa *et al.* 1994) and femoral artery (Plane *et al.* 1995).

The fact that there is still a part of L-NAME resistant relaxation in response to ACh suggests that

either NO-independent mechanism(s) participate in ACh relaxation, or that L-NAME inhibition is incomplete.

Incubation with the cyclooxygenase inhibitor indomethacin has been reported to reduce (Kato *et al.* 1990) or enhance (Zanchi *et al.* 1995) the relaxation to ACh after NO synthase inhibitors. In our vessels, however, incubation with indomethacin affected neither control nor residual relaxation after L-NAME, thus excluding the possible contribution of either vasodilatory or vasoconstrictory prostaglandins in the response.

Recently, Kitagawa *et al.* (1994) showed that endothelium-dependent relaxation to ACh in the rabbit renal artery was partially mediated by a factor different from NO, probably by an endothelium-derived hyperpolarizing factor. Similarly, both NO and a hyperpolarizing factor were shown to be released during ACh stimulation in the rat aorta and pulmonary artery (Chen and Suzuki, 1989, Vanheel *et al.* 1994). A different contribution of these factors to relaxation might then explain the different degree of reduction of ACh-induced relaxation after L-NAME in the aorta and the pulmonary artery.

Although Zanchi *et al.* (1995) demonstrated the persistence of NO synthase inhibition *in vitro* after chronic L-NAME treatment of animals, Deng *et al.* (1993) explained the incomplete inhibition of ACh relaxation by washing off L-NAME from the preparation in the course of the experiment. Our results obtained after acute additional administration of high L-NAME support the idea that at least a part of the persisting relaxation to ACh might be due to incomplete inhibition of NO.

Although L-arginine is a substrate for endothelial NO synthase (Palmer *et al.* 1988), the production of NO did not reach the control level as measured by control ACh relaxation in individual vessels. This may either be the result of the persisting inhibitory effect of L-NAME, or of the absence of L-glutamine in the incubation medium that has recently been shown to be important for raising NO production after administration of L-arginine (Arnal *et al.* 1995).

The results concerning the role of endothelium in β-adrenoceptor-mediated relaxation are controversial. While Gray and Marshall (1992) reported that relaxation to isoprenaline in the rat aorta was entirely endothelium-dependent, Eckly *et al.* (1994) documented endothelium-independent isoprenaline relaxation in the aorta of the same animal species. Our results are in good agreement with the latter study as well as with that of Fineman *et al.* (1991) who found no change in vasodilation to isoproterenol in pulmonary vasculature of intact lambs after N^{ω}-nitro-L-arginine treatment.

Relaxation to sodium nitroprusside was not significantly influenced by chronic L-NAME treatment. The result indicates that the reduced relaxation to ACh after L-NAME was not a consequence of impaired responsiveness of vascular smooth muscles in hypertensive arteries.

The potentiation of maximum force and sensitivity was found after endothelium removal (Holécyová *et al.* 1993, Atkinson *et al.* 1994) as well as after NO inhibition (Vo *et al.* 1991, MacLean *et al.* 1993), indicating that NA-induced release of NO attenuates the vasoconstrictor response to the agonist. In the rabbit pulmonary artery, augmentation of the contractile response to NA by L-NAME has been shown to involve activation of both α_1- and α_2-adrenoceptors (MacLean *et al.* 1993). A similar mechanism might also operate in the renal artery. Differences in the effect of L-NAME on maximum NA-induced contractions might also be due to a different contribution of NO to relaxation or inhibition of NA-induced contractions in individual vessels as has already been discussed above.

Our results demonstrate that NO plays a similar, although quantitatively different, role in the modulation of vascular responses in both high- and low-pressure vessels. The fact that the relaxation to ACh was attenuated in both types of vessels, independently of blood pressure in the vessels *in vivo*, indicates that NO restriction rather than elevated blood pressure plays a decisive role in impaired vascular responsiveness. In support of this statement another finding from our laboratory demonstrated that the relaxation to Acetylcholine in spontaneously hypertensive rats of the same age as ours is unchanged (Török *et al.* 1995). The reduced availability of NO may induce an imbalance between vasodilatory and vasoconstrictory responses. This, together with the observed exaggerated constriction to NA, may play an important role in the development of myocardial fibrosis observed in this type of hypertension (Pecháňová *et al.* 1995), as well as in the development of hypertension itself.

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