Long-term Nitric Oxide Synthase Inhibition and Distensibility of Carotid Artery in Intact Rats

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Abstract The goal of the present study was to evaluate the effect of long-term nitric oxide synthase inhibition by N^ω-nitro-L-arginine-methyl ester (L-NAME) on the morphology and viscoelastic properties of the carotid arteries in rats. Twelve-week-old Wistar-Kyoto rats were treated for 6 weeks with either the nitric oxide synthase inhibitor L-NAME (0.4 g/L in drinking water; L-NAME rats, n=13) or tap water (control rats, n=13). Age-matched spontaneously hypertensive rats (SHR, n=14) received tap water for the same period. The internal diameter of the common carotid artery was measured continuously with an echo-tracking device with the rats under anesthesia with halothane. Intra-arterial pressure was monitored on the contralateral side. L-NAME rats exhibited arterial pressures similar to those of SHR. The distensibility-pressure curve determined in L-NAME rats was a direct continuation of that obtained in control rats. In contrast the distensibility in SHR was increased (P<.01, SHR versus L-NAME rats). Carotid artery cross-sectional area and left ventricular weight index were increased similarly in SHR and L-NAME rats compared with control rats. Thus the hypertension caused by long-term nitric oxide synthase inhibition was not associated with the increased arterial distensibility observed in SHR despite similar blood pressure elevations, similar arterial hypertrophy, and consequently similar wall stress. This suggests a role for nitric oxide in regulating the mechanical behavior of arteries exposed to high blood pressure.

Key Words • ultrasonography • hypertrophy, vascular • heart hypertrophy • nitric oxide

The close correlation between vascular structure and blood pressure is well known,1 and there is strong evidence that blood pressure plays a major role in vascular remodeling,2 but growth factors might also be involved.3 At the level of conductance arteries, a striking increase in arterial distensibility and compliance has been reported in hypertensive animals4-6 and humans7-10 compared with normotensive counterparts. In the presence of increased wall thickness, increased distensibility can be achieved only through concurrent changes in composition or organization of the arterial wall.11 Recently the endothelium has been recognized to play a pivotal role in the regulation of vascular tone,12 but it is still unknown whether it also has an impact on the mechanical properties of the arterial wall.

The aim of the present study was to investigate whether the production of nitric oxide (NO) by the endothelium has an influence on the viscoelastic properties of the arteries. For this purpose we treated Wistar-Kyoto (WKY) rats for 6 weeks with the NO synthase inhibitor N^ω-nitro-L-arginine-methyl ester (L-NAME), which produced sustained hypertension. These animals were compared not only with normotensive WKY rats (controls) but also with age-matched spontaneously hypertensive rats (SHR) displaying the same degree of hypertension as L-NAME–treated rats (L-NAME rats). The distensibility-pressure curve of the common carotid artery was established in intact animals by using an echo-tracking device.13-15 Morphometric examination was also carried out for determination of intima-media thickness (IMT) and cross-sectional area (CSA) of the carotid artery.

Methods

Twelve-week-old male SHR and normotensive WKY control rats were obtained from Iffa-Credo. They were housed for 6 weeks at a constant temperature of 23°C. Ordinary rat chow (UAR, A04) containing 100 μmol Na/g and drinking fluid were provided ad libitum. SHR (n=14) and WKY rats (n=13) received tap water for 6 weeks. Additional WKY rats (n=13) were administered L-NAME (0.4 g/L) (Sigma) in their drinking water for the same period. Animal care, surgical preparation, and experimental procedures were approved by our institution’s ethics committee for animal experiments. The characteristics of the three groups of animals are summarized in Table 1.

On the day of the experiment, anesthesia was induced and maintained with halothane (Halothane B.P., Arovet AG) at a concentration of 1.5%. The right common carotid artery was cannulated with a catheter (PE-50, Portex) filled with heparinized 0.9% NaCl solution. Intra-arterial pressure and heart rate were monitored as described previously by using a computerized data acquisition system.16 The internal diameter (ID) of the left common carotid artery was measured simultaneously by using an A-mode ultrasonic echo-tracking device (Diarad, Asulab). This device, which has a precision close to 1 μm, has been used in humans and animals.5,10,17 For the recordings a 10-MHz probe was placed perpendicularly over the artery without direct contact with the skin. Doppler was used to guide the probe, and ultrasonic gel was used for signal transduction.

Ten successive diameter-pressure recordings were determined for each animal in a given 5-minute period and then averaged for analysis. The simultaneous arterial diameter and blood pressure measurements were processed on-line to calculate a diameter-pressure relation, which was converted into
cross-sectional compliance- and distensibility-pressure curves. These curves fit best with an arc tangent function. Cross-sectional compliance (C) in the case of a cylindrical vessel can be expressed by $C = DS/DP$ where DS is the change in arterial cross section, and DP is the change in blood pressure. Arterial distensibility (D) is the compliance value normalized for cross section (S). It is expressed as $D = (1/S) \times DS/DP$.

Once the measurements were completed, the animals were killed with a lethal dose of pentobarbital (90 mg/kg IV; CHUV). The left common carotid artery was excised, fixed in 4% paraformaldehyde. The left ventricle was then dissected and stained by hematoxylin and eosin. Histometric measurements were performed with a laser-scanned confocal microscope (MRC 500 confocal imaging system, Bio-Rad). The scanner and detectors were attached to an inverted microscope (Diaphot, Nikon). The IMT and ID measurements were performed with a 200-fold magnification by two independent investigators in a blinded fashion. The measurements carried out on two carotid sections and on six fields per section were averaged. The intima-media CSA of the fixed arteries was determined according to the following formula:

$$CSA = \pi (\text{Internal Radius} + \text{IMT})^2 - \text{Internal Radius}^2$$

The heart was also obtained after killing the animals. It was washed with phosphate-buffered saline and fixed with 4% paraformaldehyde. The left ventricle was then dissected and weighed.

**Statistical Analysis**

The between-group comparisons of body weight, left ventricular weight index, ID of the carotid artery in vitro, isobaric ID of the carotid artery in vivo, CSA, IMT, blood pressure, and heart rate measurements were made by one-way analysis of variance followed when required by Fisher's test for least significant differences. The diameter- and distensibility-pressure curves were established within operating pressures, with the upper and lower limits representing the mean systolic and diastolic values for the group, respectively. For statistical evaluation of the diameter- and distensibility-pressure curves, two different approaches were used. The curves were first compared with a multivariate analysis, based on Hotelling's $T^2$, that considered diameter and distensibility values at three arbitrarily defined blood pressures in the proximity of measured pressures (120, 160, and 200 mm Hg). The diameter- and distensibility-pressure curves were also statistically analyzed by comparing areas under the curve of the two groups with a Student's $t$ test for unpaired data. The areas under the curves were calculated taking the limits of the overlapping blood pressure ranges between both groups as the lower and upper limits.

**Results**

Table 1 gives the characteristics of the three groups of rats. L-NAME rats gained significantly less weight ($P<.001$) than the control rats or SHR. While the rats were under anesthesia, intra-arterial pressure was significantly lower in control rats than in the two other groups ($P<.01$). No difference was found, however, between L-NAME rats and SHR that received tap water as drinking fluid. No difference in heart rate was observed between the groups. The ID of the common carotid artery as measured by the echo-tracking device was similar in the three groups of rats as determined at a pressure of 125 mm Hg, ie, at an operational pressure for all animals. The arterial diameter-pressure curves showed the expected increase in arterial diameter with the level of pressure (data not shown).

Table 2 gives the results of the morphometric studies. The ID of the common carotid artery was significantly larger in SHR than in control rats ($P<.05$), with L-NAME rats exhibiting intermediate values. Both IMT and CSA were markedly increased in the two groups of hypertensive animals compared with control rats ($P<.01$). For both parameters, however, there was no difference between L-NAME rats and SHR. A similar pattern was observed for left ventricular weight index, with the difference between the normotensive control rats and the hypertensive rats being most pronounced in the SHR ($P<.01$ versus control rats).

The arterial distensibility-pressure curves established in the intact rats are shown in the Figure. The curve for L-NAME rats was a direct continuation of that observed in normotensive control rats. In contrast there was a significant upward shift in SHR, indicating increased distensibility for a given level of pressure. The difference was significant whether assessed by comparison of the area under the curve ($P<.01$) or by analysis of variance ($P<.05$).

**Discussion**

Long-term inhibition of NO synthase induces sustained hypertension in animals, and these hypertensive
animals appear to be prone to developing cerebrovascular insults, suggesting an inappropriate response of arteries to the increase in blood pressure. The aim of the present study was to evaluate the effect of long-term NO synthase inhibition by L-NAME on the geometry, morphology, and viscoelastic properties of the common carotid arteries in rats. After 6 weeks of treatment with L-NAME, these animals exhibited blood pressures similar to those of SHR with an unblocked NO synthase. As anticipated, control rats had significantly lower pressures. Despite the higher blood pressure and increased carotid wall thickness of L-NAME rats, no change in arterial wall mechanical properties was observed compared with the normotensive control rats. This is in contrast to the equally hypertensive SHR, which had a similar degree of arterial wall thickening. As reported previously, these SHR exhibited markedly increased arterial distensibility.

The three groups of animals had overlapping operating pressures over a small range at 125 mm Hg. At this level of blood pressure, the diameters of the common carotid arteries determined in vivo were similar in the three groups of animals (Table 1). This suggests that the level of blood pressure per se is the main determinant of the ID of such an elastic artery. Actually, IMT and CSA were increased to a similar degree in the two groups of hypertensive rats, independent of the activity of NO synthase. In this study, the carotid arteries were not pressurized before being fixed with paraformaldehyde. This explains the reduced ID observed in vitro in all groups. Nevertheless, this should not influence our measurement of CSA, unless a longitudinal retraction of variable degree among the models has occurred. There is, however, no reason to believe that the magnitude of such a phenomenon would be different in the three groups of animals. Structural vascular changes are known to be present very early in the development of genetic hypertension in rats, even before achievement of a sustained blood pressure elevation. Thus in L-NAME rats the arterial hypertrophy must have developed over a shorter period of time than in SHR. Whether this is the result of factors other than the rapid blood pressure elevation remains to be demonstrated. One might speculate that there is an enhanced contribution of growth factors under NO synthase inhibition. We have previously found very high plasma renin and catecholamine levels in rats that were treated for 6 weeks with L-NAME. Both angiotensin II and norepinephrine are well-established promoters of vascular growth. Conceivably the effect of these vasoconstrictor substances on the morphology of the vascular wall was amplified during NO synthase inhibition. Furthermore, NO has been shown to inhibit vascular smooth muscle cell proliferation.

The body weight gain was reduced in L-NAME-treated rats. This is not surprising because similar findings have been reported with similar doses of L-NAME. In our experience, the 0.4-g/L dose of drinking water for L-NAME rats provides efficient inhibition of NO synthase, as assessed by the persistent blockade of the vasodilator response to acetylcholine in isolated mesenteric arteries taken from rats that received L-NAME in vivo under conditions similar to those described here.

A striking feature of the present study is the discrepancy of arterial distensibility between the two hypertensive groups. This difference exists despite similarly elevated blood pressures and increased wall thicknesses. Such findings imply a differential wall structure, composition, and/or organization. This therefore suggests a key role for NO in modulating the vascular remodeling. The true relevance of the enhanced arterial distensibility seen in SHR is unknown. Because of the increased buffering capacity of their large arteries, these animals might be better protected against high blood pressure than L-NAME rats, although the wall stress could be normalized in both hypertensive groups via the arterial wall thickening.

Ventricular hypertrophy allows restoration of normal cardiac wall stress in the face of hypertension. The increased cardiac mass found in the two groups of hypertensive rats therefore could be anticipated. It must be pointed out, however, that divergent results have been published with respect to the development of cardiac hypertrophy under prolonged NO synthase inhibition. Our findings are consistent with those reported recently in rats that were treated for 4 weeks with L-NAME. In that study, there was some indication of a link between the degree of activity of the renin-angiotensin system and the severity of cardiac hypertrophy. Previous experiments performed in our laboratory have revealed very high renin levels in rats that were treated with L-NAME for 6 weeks.

In conclusion, we have shown that hypertension induced by NO synthase inhibition for 6 weeks is associated with carotid artery and cardiac hypertrophy. The degree of hypertrophy is similar to that of age- and blood pressure-matched SHR. However, for an equal degree of arterial wall thickening, the structural changes induced by L-NAME are not associated with the enhanced arterial distensibility characterizing the common carotid artery of SHR. These data therefore suggest a role for NO in modulating the viscoelastic properties of large arteries exposed to high blood pressure.

**Plot of relation between intra-arterial pressure and distensibility of common carotid artery in Wistar-Kyoto (WKY) control rats, N^2-nitro-L-arginine methyl ester (L-NAME)-treated WKY (WKY+L-NAME) rats, and spontaneously hypertensive rats (SHR).**
References

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