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Diminished Contractile Responses of Isolated Conduit Arteries in Two Rat Models of Hypertension

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Abstract

Hypertension is accompanied by thickening of arteries, resulting in marked changes in their passive and active mechanical properties. The aim of this study was to demonstrate that the large conduit arteries from hypertensive individuals may not exhibit enhanced contractions *in vitro*, as is often claimed. Mechanical responses to vasoconstrictor stimuli were measured under isometric conditions using ring arterial segments isolated from spontaneously hypertensive rats, N^{ω}-nitro-L-arginine methyl ester (L-NAME)-treated Wistar rats, and untreated Wistar rats serving as normotensive control. We found that thoracic aortas from both types of hypertensive rats had a greater sensitivity but diminished maximal developed tension in response to noradrenaline, when compared with that from normotensive rats. In superior mesenteric arteries, the sensitivity to noradrenaline was similar in all examined rat groups but in L-NAME-treated rats, these arteries exhibited decreased active force when stimulated with high noradrenaline concentrations, or with 100 mM KCl. These results indicate that hypertension leads to specific biomechanical alterations in diverse arterial types which are reflected in different modifications in their contractile properties.

Key Words: contraction, isometric force, large arteries, noradrenaline, NO-synthase blockade, smooth muscle, spontaneously hypertensive rat

Introduction

Hypertension is associated with several structural and functional alterations in the cardiovascular system representing adaptations to the hemodynamic overload. One of the most prominent changes is thickening of arteries leading to the normalization of tensile forces in their walls. This process involves both smooth muscle hypertrophy as well as accumulation and reorganization of passive elements, namely the extracellular matrix containing elastin and collagen fibers. Arterial thickening is often associated with increased stiffness, which is particularly important in large conduit arteries when considering their buffering role during cardiac cycle. The rigid wall restrains functions of these arteries as elastic reservoirs which undergo large volume changes with little change in pressure (30). In the present study, we tried to show that the altered mechanical properties of arterial wall in hypertension may affect not only the passive functions of large arteries (buffering of stroke volume and pulse wave propagation) but also the manifestation of active force generated by arterial smooth muscle. This second characteristic is often studied in *in vitro* conditions on the ring arterial preparations in which the mechanical and pharmacological properties of vascular smooth muscle during normal and pathological conditions are investigated (2, 27, 28). In this regard, more suitable seems to be the small resistance arteries in which the muscle component and its func-

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tions are particularly dominant. However, as stated by Mulvany and Halpern (22), by reason of methodological limitations, most of this information until the mid-1970s was obtained only from large elastic vessels, animal aorta being the most examined. Despite the fact that there are many techniques for investigating much smaller vessels today [with internal diameters down to around 100 μm; (22)], large arteries are still regularly being used in the study of functional and morphological alterations accompanying various cardiovascular diseases (17, 27).

Results obtained from hypertensive animal models clearly showed hypertrophy of medial layer in the large arteries of the animals studied (12, 14). It is appropriate to presume that the presence of the greater smooth muscle mass in hypertensive arteries should produce greater active force in response to vasoconstrictor stimuli. On the other hand, one could also envisage that the smooth muscle cells, being embedded in the rigid extracellular materials of the arterial wall, are not able to sufficiently manifest their contraction potential. In other words, the force generated by smooth muscle is not transferred to other structures within the arterial wall and, therefore, may not be recorded under particular experimental conditions. This idea was developed during many of our past measurements when we regularly found diminished contractile responses of conduit arteries isolated from hypertensive rats in comparison with that from normotensive rats, examined under similar experimental circumstances (unpublished data).

For many investigators, it is hardly acceptable that hypertensive individuals could have smaller arterial contractions. In this study, we aimed to clearly characterize the differences in sensitivity and maximal contractile responses of selected conduit arteries to vasoconstrictor stimuli in normotensive and hypertensive rats.

Materials and Methods

Experimental Animals

The animal protocols used in this study were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, and approved by the Animal Health and Welfare Division of the State Veterinary and Food Administration of the Slovak Republic. All rats were housed at 22-24°C on a 12:12-h dark-light cycle (06.00-18.00 h lights on) and maintained on a standard laboratory rat chow and tap water *ad libitum*. In this experiment, twelve-week-old male rats were used: untreated Wistar rats (normotensive control); Wistar rats in which hypertension was induced by long-term administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME, an inhibitor of NO synthase enzyme, obtained from Sigma-Aldrich Chemie GmbH, Steiheim, Germany) at 40 mg/kg/day for 6 weeks; spontaneously hypertensive rats (SHR). Systolic blood pressure was measured non-invasively in conscious rats by the tail-cuff method.

Functional Studies on Isolated Arteries

Functional studies were performed on isolated thoracic aorta and superior mesenteric artery. After sacrificing the rats under CO_2 anesthesia, the arteries were carefully removed, cut into rings of 3.0-3.5 mm in width and suspended in 20 ml organ baths filled with oxygenated (95% O_2 + 5% CO_2) modified Krebs solution maintained at 37°C. The Krebs solution had the following composition (all in mM): NaCl 118, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, $CaNa_2$.EDTA 0.03 and ascorbic acid 0.55. The arterial rings were set up for isometric tension recording using a force-displacement transducer Sanborn FT 10 (Sanborn, Baltimore, USA). The preparations were equilibrated under a resting tension of 10 mN for 60-90 min, and the solution was changed every 15 min.

In the presence of endothelium, the sensitivity and contractile force development in response to vasoconstrictors were assessed. Responses to cumulatively applied noradrenaline (in the range of concentrations from 10^{-10} to 10^{-5} M) were measured in both arteries; moreover, in superior mesenteric artery, contraction to 100 mM KCl was determined.

Data Analysis

The results are presented as means ± SEM. Contractile responses are expressed in absolute (in mN) or relative (in % of maximal contraction) values. Statistical evaluation was carried out by using one-way analysis of variance (ANOVA). The results were considered to be significant when $P < 0.05$.

Results

The experiments performed in this study were based on isometric recording of smooth muscle tension in arterial preparations *in vitro*. Using this method, we measured the mechanical responses to vasoconstrictor stimuli in thoracic aorta and superior mesenteric artery and compared them between normotensive and hypertensive rats.

Blood pressure in male 12-week-old SHR (185.5 ± 4.4 mmHg) was significantly higher than in age-matched Wistar rats (120.0 ± 0.4 mmHg) ($P < 0.001$). Treatment of Wistar rats with L-NAME led

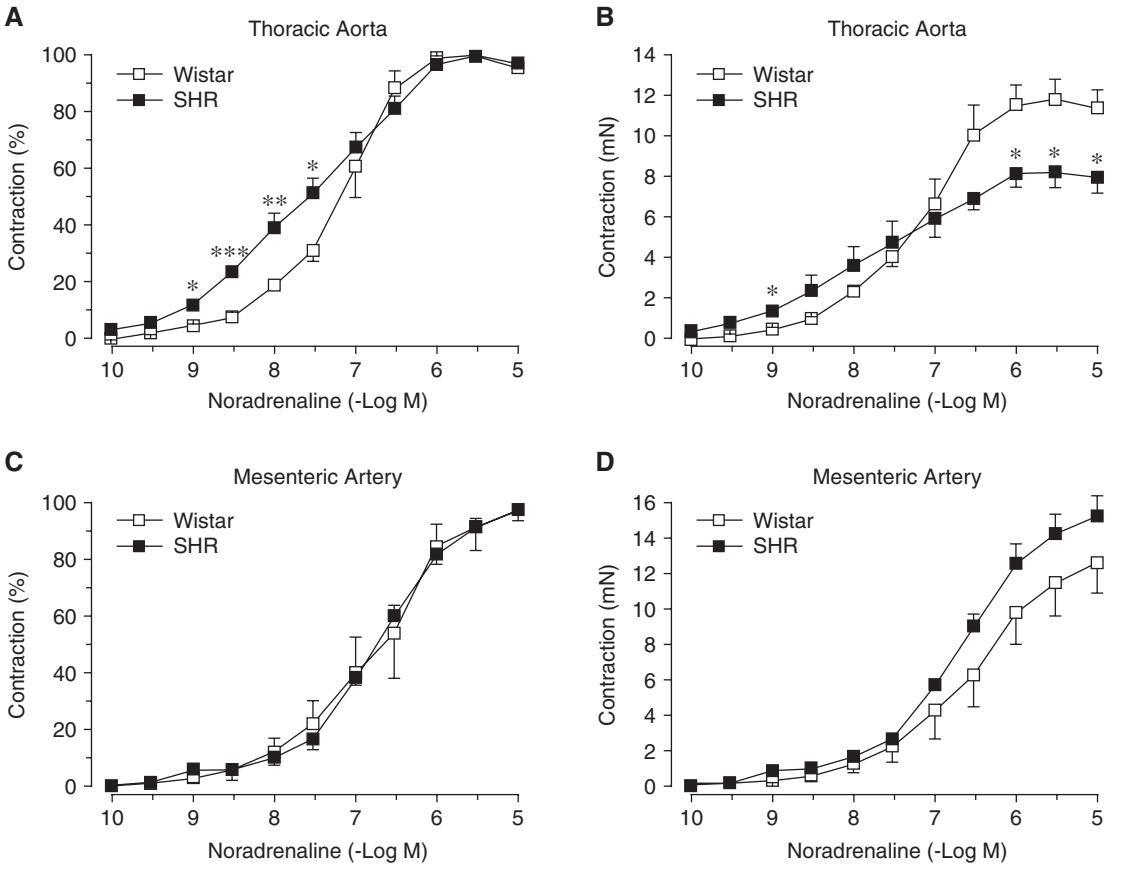

Fig. 1. Comparison of noradrenaline-induced contractile responses in conduit arteries from normotensive Wistar rats and spontaneously hypertensive rats (SHR): Line graphs show the concentration-response curves in relative (A, C) and absolute (B, D) values for thoracic aorta (A, B) and superior mesenteric artery (C, D). Data are presented as means ± SEM obtained from six to ten different animals in each experimental group. *, ** and *** indicate $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, in SHR compared with the normotensive rats.

to the increase in blood pressure to 174.6 ± 2.1 mmHg ($P < 0.001$).

Thoracic aortas from SHR exhibited greater sensitivity to low noradrenaline concentrations but the absolute maximal contractile force was markedly reduced comparing to the normotensive Wistar rats (Fig. 1, A and B). Superior mesenteric arteries of both rat groups produced very similar contractile responses to noradrenaline (Fig. 1, C and D) as well as to the increase of potassium ions in the Krebs solution to 100 mM (10.34 ± 0.69 mN in Wistar rats and 11.57 ± 0.21 mN in SHR).

In Wistar rats treated with L-NAME, we observed a striking reduction of arterial contractions to vasoactive drugs comparing to the age-matched untreated controls (Fig. 2, B and D). In thoracic aortas, the relative dose-response curve was shifted to the left but the absolute values showed apparent decreases in response to high noradrenaline concentrations (Fig. 2, A and B). In superior mesenteric arteries, maximal contractions to noradrenaline (Fig. 2D) and to 100 mM KCl (10.44 ± 0.87 mN in untreated and 7.31 ± 0.88 mN in L-NAME-treated rats; $P < 0.05$) were also diminished.

Discussion

In the present study, we attempted to demonstrate that the large conduit arteries from hypertensive rats may not exhibit enhanced contractions *in vitro*, as is often claimed. In the two types of experimental hypertension used (genetic – spontaneous, and exogenously induced – due to long-term L-NAME administration), morphometry of large arteries showed increases in wall thickness and cross sectional area compared with age-matched normotensive Wistar rats (11, 28). This finding can be related to structural adaptation to increased wall (hoop) stress at sustained high blood pressure. However, our presented results revealing the decreased contractile responses of arteries of hypertensive rats are more difficult to explain.

Some authors have suggested that the reduction of arterial contractile responses in hypertensive rats

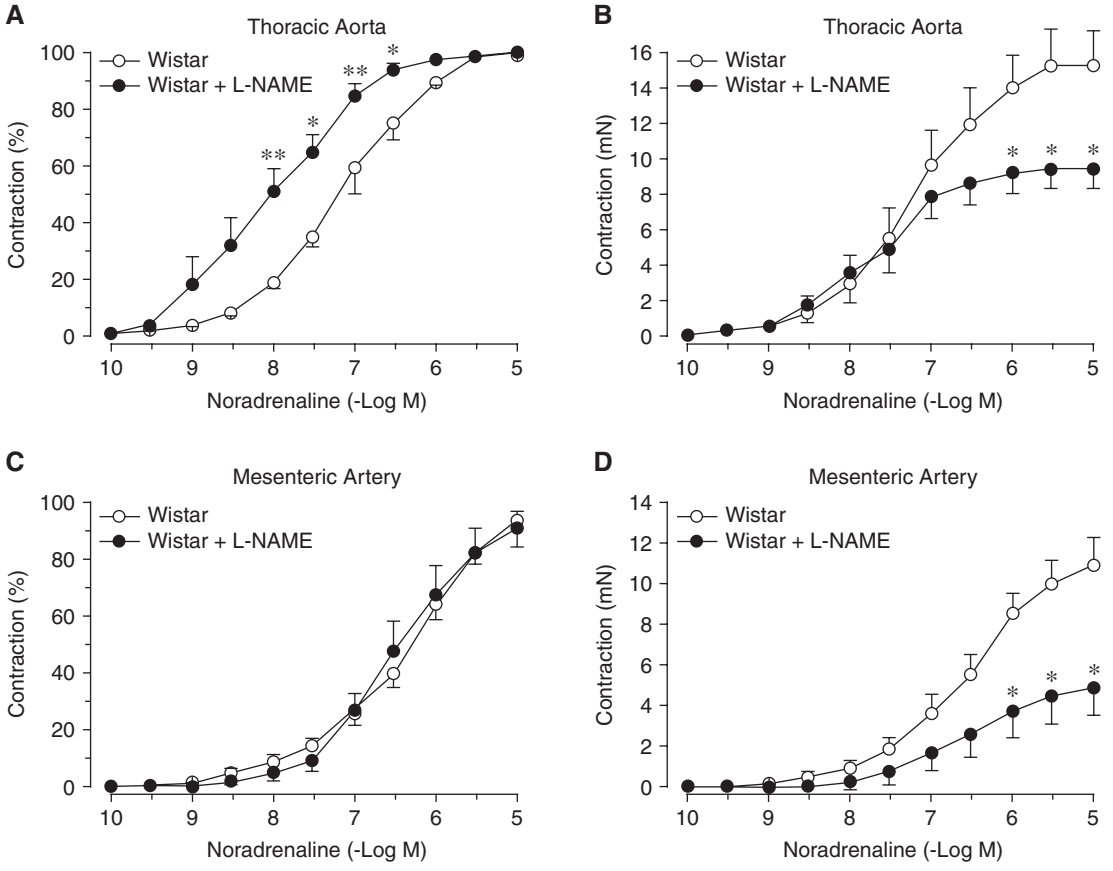

Fig. 2. Comparison of noradrenaline-induced contractile responses in conduit arteries from normotensive Wistar rats and rats made hypertensive with L-NAME treatment: Line graphs show the concentration-response curves in relative (A, C) and absolute (B, D) values for thoracic aorta (A, B) and superior mesenteric artery (C, D). Data are presented as means ± SEM obtained from six to ten different animals in each experimental group. * and ** indicate $P < 0.05$ and $P < 0.01$, respectively, in L-NAME-treated rats compared with untreated rats.

is due to desensitization of smooth muscle to constrictoric stimuli, such as down-regulation of the receptor system or inhibition of the contractile apparatus, in order to adapt to a higher sympathoadrenergic stimulation and to compensate for the abnormal vascular resistance (7, 31). However, in our measurements on conduit arteries from the SHR and L-NAME-treated rats the sensitivity to noradrenaline did not seem to be blunted because the contractile responses to lower concentrations of noradrenaline were not diminished. Moreover, in thoracic aortas of these rats the relative dose-response curves were shifted to the left indicating that the arteries were rather sensitized and poised to contract in response to adrenergic stimulation. This effect could be partially explained by the damaged function of their endothelium, particularly in rats treated with L-NAME, in which the endothelial production of the main vasodilator substance, nitric oxide (NO), is inhibited and thus the negative regulation of vascular tone is disrupted. On the other hand, the higher sensitivity of SHR aortic smooth muscle to low noradrenaline concentrations could be the consequence of inherent alterations in myocyte calcium handling, including enhanced calcium entry and augmented calcium release from sarcoplasmic reticulum (3, 4), or due to differences and abundances in adrenoreceptor subtypes (9).

Our observations, therefore, suggest that in hypertensive conduit arteries the sensitivity of smooth muscle to adrenergic stimuli is rather enhanced but the maximal contractile force in response to high noradrenaline concentrations is diminished. One of the explanations for that finding could be the fatigue of vascular smooth muscle due to its excessive activity in hypertensive state, leading to faster weakening of contractile responses (15). On the other hand, there may be some structural limitations which prevent the muscle cells to contract effectively. This hypothesis could be related to the phenomenon of arterial rigidity and stiffness which is often described in hypertensive states (25). In the literature there are several reports concerning increased stiffness of conduit arteries in hypertensive rats (1, 5, 8) but these alterations are

apparently different from changes observed in human aging and hypertension. In SHR, aberrations in arterial morphology and biomechanical properties appear to be inherited because these alterations are already present in the juvenile period, *i.e.*, before the pathological increase in blood pressure (1, 24, 29). The structural aberrations could specifically alter the contractile properties in particular vascular beds and may lead to different abnormalities in various arterial types. In normal aorta, there is a typical lamellar structure of the medial layer, consisting of marked elastin-containing sheets between which are collagen fibers, thin layers of proteoglycan-rich extracellular matrix, and smooth muscle cells (30, 32). In pathological conditions, including abnormal depositions or arrangements of these components, the extracellular material enclosing muscle cells may act as a "concrete encasement" and compromise the contractile functions of the smooth muscle. On the contrary, rat superior mesenteric artery is an elasto-muscular vessel that is structurally and functionally different from the aorta (21). Moreover, mesenteric artery is characterized by relatively rich sympathetic innervation which is particularly pronounced in SHR (18, 20, 26). It was found that the perivascular sympathetic nerves strongly promote the differentiated contractile phenotype of vascular smooth muscle cells and suppress the production of extracellular matrix components (6). This could contribute to the fact that this artery behaved differently from aorta and had no tendency to diminution of contractility in SHR in our study.

In contrast to SHR, reduction in arterial contractile responses in rats made hypertensive with L-NAME treatment is present more globally in the vasculature (7, 17). This finding may seem unexpected not only because of the hypertrophied arterial walls in these rats but also due to their markedly impaired vasorelaxation (28). Inhibition of NO synthesis with L-NAME causes increased responsiveness of smooth muscle to constrictoric stimuli; this effect is clearly seen in acute experiments when L-NAME is applied directly on arterial preparations and causes potentiation of contractile responses (13). But in chronic conditions, when L-NAME is delivered to the organism for several weeks (especially in higher doses), the sustained inhibition of NO synthase has some additional long-term consequences on cardiovascular functions. Besides the adaptation to severe systemic hypertension due to suppressed NO-dependent vasorelaxation, long-term NO deficiency alone induces structural alterations in cardiovascular tissues like fibrotic lesions (19, 23), lipid deposition in arterial wall (33), or functional changes of vascular smooth muscle cells resulting from decreased contractile protein expression or reduction in extracellular Ca^{2+} influx (17, 33) which indicate the loss of arterial contractile properties. This may affect smooth muscle function either directly, or also through the structural changes in the arterial wall, which constrain the transfer of muscular generated force, as was described above.

Although in our experiments we constantly observed diminished arterial contractile responses in SHR and L-NAME-treated rats, several studies of other authors reported opposite findings (2, 10, 16). The discrepancies may reflect the different methodological approaches, arterial types used, or the genetic variability in SHR and different genetic backgrounds of normotensive rats made hypertensive with L-NAME.

The results of this study cast new insights into the assessment of arterial contractility in normotensive and hypertensive rats. We conclude that the hypertension in SHR and in L-NAME-treated rats, although accompanied with marked hypertrophy of the arterial wall, may not be exclusively associated with enhanced contractile responses. This could be taken into accounts particularly in large elastic arteries with respect to their specific structure and functions, which may be the cause of different alterations in response to sustained elevation in blood pressure.

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