Mechanical Strength of the Isolated Carotid Artery in SHR

Geraldine Cohuet, Pascal Challande, Mary Osborne-Pellegrin, Silvia M. Arribas, Anna Dominiczak, Huguette Louis, Stéphane Laurent, Patrick Lacolley

Abstract—We have previously reported an adaptation of arterial wall elasticity in spontaneously hypertensive rats (SHR) that involves an increase in both fibronectin/α5β1-integrin complexes and smooth-muscle elastic lamellae connections. We examined the mechanical strength (MS) of the carotid artery in relation to its elastic properties, its elastin/collagen content, and the structure of the internal elastic lamina. MS was defined as the in vitro intraluminal pressure and wall stress that produces rupture of the vascular wall. Intact carotid arteries from 3-month-old normotensive rats (Wistar-Kyoto, WKY) and SHR were cannulated on a specially designed device and adjusted to their in situ length. A slowly increasing static pressure was applied until wall rupture occurred to determine the static mechanical behavior and MS. Static elasticity was similar in SHR and WKY, as were the rupture pressure (2740±90 versus 2740±40 mm Hg) and wall stress at rupture (11.5±1.0 versus 12.8±0.4 MPa), indicating equivalent MS in both groups. Histological examination showed several wall ruptures and dissociation of lamellar units that did not differ significantly between the 2 groups. Confocal microscopy showed that the size of fenestrations of the internal elastic lamina and the fraction of area occupied by them were reduced 3-fold in SHR. We have demonstrated that static elasticity of the arterial wall and mechanical strength are similar in carotid arteries from SHR and WKY. (Hypertension. 2001;38:1167-1171.)

Key Words: arteries ■ collagen ■ hypertension, experimental ■ remodeling

In elastic arteries of spontaneously hypertensive rats (SHR), there is no increase in wall stiffness despite the increase in wall thickness, a sign of mechanical adaptation of the arterial wall to the higher level of stress.1-4 We have previously demonstrated that structural modifications such as an increase in fibronectin α5β1-integrin complexes of the media and an increase in connections of muscle cells to elastic lamellae may participate in this mechanical adaptation in hypertension.2,5 More recently, we have reported that the size of fenestrations of the internal elastic lamina (IEL) and the fraction of IEL area occupied by them were decreased in stroke-prone hypertensive rats (SHRSP), without change in relative elastin content, compared with Wistar-Kyoto rats (WKY), indicating a reduction of the stress concentration phenomenon in the IEL.6 We have suggested that all these adaptive structural changes may alter the distribution of the wall stress through the media and contribute to the maintenance of the normal level of vascular wall elasticity despite the increase in stress caused by the higher physiological pressure.

Because a greater number of cell-matrix attachment sites in the media and a limitation of stress concentration in the IEL can favor the mechanical integrity of the arterial wall at elevated arterial pressures, it may be particularly informative to determine the mechanical strength (MS) of the vascular wall of SHR at high levels of stress. The mechanical strength was defined as the in vitro intraluminal pressure and wall stress producing the rupture of the vascular wall. This parameter could play an important role in protecting the arterial wall against the effects of fatigue, such as deterioration of elastic fibers, leading to excessive fragilization of the arterial wall. In pathology, MS may provide additional information concerning the onset of arterial complications, such as dissection and rupture of the vascular wall observed in various diseases of connective tissue.

The direct measurement of the MS of elastic arteries in SHR has never been performed. The only published study concerns the determination of the longitudinal passive stress-strain curves in relation to elastin and collagen contents and shows an increase in the maximum stress in 12-week-old SHR compared with WKY without changes in elastin/collagen ratio or in collagen-advanced glycation end products.7

Therefore, our objective was to evaluate in SHR and WKY the MS of the carotid artery (CA) in relation to the structure of the IEL, a representative component of the arterial elastic network, with the use of laser scanning confocal microscopy (LSCM). In parallel, the aortic elastin and collagen contents were determined in the 2 groups of rats, assuming that these values may reflect the situation in the CA.

Received April 28, 2001; first decision June 18, 2001; revision accepted July 6, 2001.
From the “Institut National de la Santé et de la Recherche Médicale,” Inserm EMI 0107 (G.C., H.L., S.L., P.L.), U460 (M.O.P.), Paris, LMP, Université Paris VI (P.C.), France; Departamento de Fisiologia, Facultad de Medicina, Universidad Autónoma de Madrid (S.A.), Spain; and the Department of Medicine and Therapeutics, University of Glasgow (A.D.), Scotland, United Kingdom.
Correspondence to Patrick Lacolley, MD, PhD, Inserm EMI 0107, 15 rue de l’École de Médecine, 75270 Paris Cedex 06, France. E-mail lacolley@ccr.jussieu.fr
© 2001 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org
Methods

Animals
The study was performed in 3-month-old male SHR (n=16) and WKY (n=25) as controls (Iffa-Credo, France). All procedures were in accordance with institutional guidelines for animal experimentation.

Elasticity and Mechanical Strength of the CA
A 1-cm segment of the CA free of collaterals was carefully dissected and placed in warm (37°C) Krebs buffer. It was then cannulated on a specially designed device and adjusted to its in situ length.

The MS was characterized by the rupture pressure and the corresponding wall stress. Thus, an increasing intraluminal static pressure was applied and continuously measured by a pressure transducer (UP4, Pioden) until rupture of the vascular wall was achieved. The internal arterial diameter was measured with an ultrasonic echo-tracking device (NIUS-01, Asulab SA). Pressure/diameter and pressure/distensibility curves were obtained during the distension of the vessel. The circumferential wall stress at which rupture occurred was calculated by using the value of medial diameter and pressure/distensibility curves were obtained during the distension of the vessel. The circumferential wall stress at which rupture occurred was calculated by using the value of medial diameter and pressure/distensibility curves were obtained during the distension of the vessel. The circumferential wall stress at which rupture occurred was calculated by using the value of medial diameter and pressure/distensibility curves were obtained during the distension of the vessel. The circumferential wall stress at which rupture occurred was calculated by using the value of medial diameter and pressure/distensibility curves were obtained during the distension of the vessel. The circumferential wall stress at which rupture occurred was calculated by using the value of medial diameter and pressure/distensibility curves were obtained during the distension of the vessel. The circumferential wall stress at which rupture occurred was calculated by using the value of medial diameter and pressure/distensibility curves were obtained during the distension of the vessel. The circumferential wall stress at which rupture occurred was calculated by using the value of medial diameter and pressure/distensibility curves were obtained during the distension of the vessel.

Quantification of Aortic Elastin and Collagen
For methodological reasons, biochemical analysis was performed on the descending thoracic aorta. As previously described, elastin and collagen were quantified on individual aortas without homogenization. Briefly, after delipidation and recording of dry weight, the extracellular proteins other than elastin were solubilized by hot 0.1N NaOH. The residue, elastin, was quantified by weighing and the collagen present in the NaOH by determining hydroxyproline after hydrolysis.

Quantification of IEL Fenestrations with Confocal Microscopy
Arteries were pressurized in vitro with 10% formaldehyde-saline solution for 1 hour at 100 mm Hg with a pressure-perfusion myograph. We used the same pressure fixation of 100 mm Hg in both WKY and SHR because the fixation at 100 mm Hg and 170 mm Hg, representing their in vivo respective mean arterial pressure, produced no change in the size of fenestrations in SHR.

The central part of each artery was opened longitudinally to study IEL fenestrations with an LSCM. We used an Olympus LSCM fitted with an argon-ion laser line (Noran Instruments) coupled to a Nikon Optiphot. Lamellae are mainly composed of elastin, which has autofluorescent properties in the band of 500/560-nm wavelength and can be detected with the 488/515-nm wavelength of the LSCM. From each vessel, 10 images were captured with a ×40 oil immersion objective (Nikon, NA 1.3). Metamorph software (Universal Imaging Corporation) was used to quantify the number and area of fenestrations in the images and fraction of wall area occupied by fenestrations.

Statistical Analysis
All values were averaged and expressed as mean±SEM. Statistical comparisons between groups were made by means of the nonparametric Mann-Whitney test. Differences were considered significant at values of P<0.05.

Results
Elasticity and Mechanical Strength
SHR had significantly lower body weight than did WKY (348±5 g versus 384±21 g, P<0.05), whereas the MCSA was significantly greater in SHR (0.138±0.011 mm²) than in normotensive rats (0.088±0.004 mm², P<0.05).

Figure 1 shows the evolution of the diameter and static distensibility when the in vitro intraluminal pressure was increased until rupture of the vascular wall was achieved. The 2 diameter/pressure curves are parallel and show no significant difference in static distensibility between SHR and WKY. Internal diameter increased by ∼20%, from 200 to 2000 mm Hg. Static distensibility markedly decreased with increasing pressure and fell to below 10% of its initial value (observed at 200 mm Hg) at 1200 mm Hg. Thus the static mechanical behavior of the CA was equivalent in the 2 groups.

The rupture pressure was similar in SHR compared with WKY (Table), whereas the wall stress calculated at the rupture pressure was slightly reduced in SHR (∼10%, NS), because of the significant increase in arterial MCSA in SHR. Thus, the MS of the arterial wall was equivalent in both groups.

The rupture of the vascular wall was confirmed by histological examination of in vitro preparations. Complete wall rupture, involving intima, media, and adventitia, occurs perpendicular to the long axis of the segment (Figure 2A and 2B). In SHR, the number of these main ruptures was not significantly different from that of WKY, and their cumulative surface area was not significantly increased (Figure 2D). The applied elevated pressures produced additional secondary, incomplete, longitudinal, or transverse ruptures involving the IEL alone or with part of the media, scattered in the carotid segment (Figure 2A and 2B). There was no significant difference in the number of secondary ruptures between SHR.
and WKY (3.8±1.5 versus 1.5±0.5). However, 5 SHR of 6 showed groups of longitudinal lesions originating from a main zone of rupture (Figure 2C), whereas no such lesions were observed in WKY. On transverse paraffin sections of artery exposed to high pressure, in SHR, areas of dissociation of lamellar units were visible (Figure 2E), whereas an intact wall structure was maintained when the artery was fixed at 100 mm Hg (Figure 2F).

Aortic Elastin and Collagen Contents

Whether expressed as a percentage of aortic dry weight or as milligrams per centimeter of aorta, total collagen was significantly lower in SHR than in WKY, whereas there was a slight but nonsignificant increase in elastin content (Table), resulting in an increased elastin/collagen ratio in SHR.

LSCM Study of Elastic Lamella Structure

Figure 3 shows the fenestrations of the IEL after fixation at 100 mm Hg in SHR and WKY. The IEL of WKY and SHR were perforated with elliptical fenestrations. The number of fenestrations per unit area was not different between the 2 strains, but the fraction of area occupied by fenestrations was reduced 3-fold in SHR compared with WKY.

Discussion

The aim of this study was to evaluate the MS of the CA in relation to its intrinsic elastic properties, the elastin/collagen content, and the structure of the IEL. The main findings in 12-week-old SHR compared with normotensive rats are (1) the MS of the vascular wall was identical; (2) the static mechanical behavior during the phase of distension (from 200 to 2000 mm Hg) was similar despite vascular wall hypertrophy and an increase in elastin/collagen ratio; (3) no significant differences in the zones of rupture and structural alterations of the vascular wall were detected; and (4) the fraction of area of the IEL occupied by fenestrations was smaller.

MS is a fundamental parameter of arterial wall mechanics, but this parameter is difficult to assess directly in intact blood vessels because it requires very high pressure levels. Using an in vitro technique, we characterized the MS of the CA in groups of SHR and WKY. This technique has been described previously in mouse lacking desmin. The circumferential stress induced by pressure is the main mechanical solicitation in normal and pathological situations. Our data indicate that the MS in a large elastic artery, the CA, was not different between normotensive rats and SHR, whether evaluated by pressure or circumferential wall stress at rupture. Up until now, the only published comparison of MS between SHR and normotensive rat arteries was made by stretching the aorta along its longitudinal axis, and no difference in the longitudinal breaking stress was reported. We can thus conclude that SHR and WKY have identical MS evaluated in longitudinal and circumferential directions. Our study shows that the values of circumferential wall stress at rupture were ≈2-fold higher than those of longitudinal stress previously reported. Even though these measurements were made in elastic arteries of different caliber (thoracic aorta versus CA), the discrepancy may be mainly attributed to the anisotropy of...
the wall related to the disposition of circular elastic lamellae alternating with parallel layers of muscle cells within the media.14,15

Elasticity is the other fundamental parameter of arterial wall mechanics. We found that elastic properties were similar in SHR and WKY in static conditions during the distending phase despite a reduction in collagen content and an increased elastin/collagen ratio, as assumed by extrapolation of results in the thoracic aorta, as it has been previously shown that relative compositions are similar in thoracic aorta and carotid artery.16 Our observation of a gradually decreasing arterial distensibility from 200 to 2000 mm Hg is related to the progressive recruitment of rigid collagen fibers and activation of cell-matrix attachment sites at high stress. The observation made by Mizutani et al7 that an increased elasticity after high-strain levels in the longitudinal direction was associated with no change in MS in SHR compared with WKY suggests that elasticity and MS are not necessarily correlated. The identical values we observed for carotid wall elasticity and MS in the circumferential direction illustrates the anisotropy of the wall.17,18 Thus our study demonstrates that the global mechanical behavior of the SHR CA is well adapted to the increase in circumferential wall stress. This finding is consistent with previous in vivo observations of an adaptation of arterial elasticity in genetic hypertension in rats and human beings.3,4,19–22

Structural examination of the CA exposed to high pressure levels revealed that the number of ruptures of the vascular wall in SHR was similar to that in WKY associated with a nonsignificant increase in the cumulative area of the main ruptures. As described by Groenink et al,11 we observed a circumferential orientation of the main ruptures. Originating from these, we observed the presence in SHR of some groups of longitudinal wall lesions, possibly indicating some structural difference in response to high stress between SHR and WKY. This observation may reflect a greater variability of the structural response at high stress, suggesting some extra-cellular matrix phenotypic changes in individual SHR. In addition to the main ruptures, secondary tears oriented longitudinally and transversally were observed in both strains, indicating that the local stresses may exceed the maximal admissible stress of the wall material in these 2 directions. However, in our experiments, we calculated a value of stress averaged over the entire arterial wall that does not reflect the local variations because of the inhomogeneity of the material.23

As previously described in SHRSP,6 we found a decrease in the size of fenestrations of the IEL and in the fraction of area occupied by fenestrations in SHR compared with WKY, with no change in elastin density. This could avoid an excessive stress concentration in the IEL,24 and thus represents an adaptive mechanism to protect the elastin network against an increased physiological mean wall stress in addition to the reinforcement of cell-matrix attachments: increased expression of fibronectin and integrin-β3 and cell-elastin connections.2,5,6,25 Our observation showing equivalent breaking stress in SHR suggests that these adaptive mechanisms do not play an important role in regulating MS. This suggests that other structural components such as the collagen network26 and other connections are involved in the determination of MS. In conclusion, we have demonstrated that static elasticity of the arterial wall and mechanical strength are similar in CA from SHR and WKY, indicating arterial wall adaptation in SHR.

Acknowledgments
This work was supported by a grant from the Institut National de la Santé et de la Recherche Médicale (INSERM No. 237/6900). We thank Aline Apartian for excellent technical assistance.

References


Mechanical Strength of the Isolated Carotid Artery in SHR
Geraldine Cohuet, Pascal Challande, Mary Osborne-Pellegrin, Silvia M. Arribas, Anna Dominiczak, Huguette Louis, Stéphane Laurent and Patrick Lacolley

Hypertension. 2001;38:1167-1171
doi: 10.1161/hy1101.095995

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/38/5/1167

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/