Resistance Artery Mechanics, Structure, and Extracellular Components in Spontaneously Hypertensive Rats
Effects of Angiotensin Receptor Antagonism and Converting Enzyme Inhibition

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Background—Altered vascular mechanics resulting from changes in collagen and integrins may influence resistance artery structure and function and, therefore, peripheral resistance and blood pressure in spontaneously hypertensive rats (SHR).

Methods and Results—Effects of age, angiotensin-converting enzyme inhibition (fosinopril, 10 to 30 mg/kg per day), and AT₁-receptor antagonism (irbesartan, 50 mg/kg per day) on vascular structure, mechanics, and composition were assessed in SHR. Systolic blood pressure was elevated in young SHR (130±2 mm Hg) compared with Wistar-Kyoto (WKY) rats (106±2 mm Hg). In adult SHR, the rise in systolic blood pressure (44±3 mm Hg) was blunted by fosinopril (18±1 mm Hg) and irbesartan (9±3 mm Hg). Lumen diameter of mesenteric resistance arteries was smaller and media/lumen ratio was greater in young and adult SHR versus WKY rats. Growth index was 24% in untreated adult SHR versus WKY rats; these values were −35% for fosinopril-treated and −29% for irbesartan-treated SHR versus untreated SHR. Isobaric wall stiffness was normal despite increased stiffness of wall components in adult SHR vessels. Irbesartan partially prevented stiffening of wall components in SHR. The collagen/elastin ratio was greater in adult SHR vessels (6.5±1.3) than in WKY (3.2±0.4) vessels. Expression of αᵥβ₃ and α₅β₁ integrins was increased in SHR aged 20 versus 6 weeks. Expression of αᵥβ₃ integrins was lower in young SHR, and α₅β₁ integrins were overexpressed in adult SHR versus WKY rats. Irbesartan and fosinopril attenuated differences in the collagen/elastin ratio and integrin expression.

Conclusions—Wall components of mesenteric resistance arteries stiffen with age in SHR. Interrupting the renin-angiotensin system has normalizing effects on integrin expression and composition, stiffness, and growth of the arterial wall.

Key Words: arteries ■ mechanics ■ remodeling ■ collagen ■ cell adhesion molecules

Resistance arteries of spontaneously hypertensive rats (SHR) undergo a combination of hypertrophic (increased media thickness and cross-sectional area) and eutrophic (decreased lumen and external diameters with unaltered media cross-sectional area) remodeling. Changes in small artery mechanics may also influence pressure-diameter relations of blood vessels. Opposing changes in wall stiffness occur with aging in different arterial beds in hypertensive rats. Increased distensibility of cerebral arterioles from 6- to 8-month-old stroke-prone SHR (SHR-SP) was absent in younger (3- to 4-month-old) SHR-SP, suggesting that prolonged hypertension increases pial arteriole compliance and decreases geometry-independent wall stiffness despite vascular hypertrophy. Decreased vascular stiffness is not a global response to prolonged hypertension. In 2-kidney 1-clip renal hypertensive rats, carotid arteries were stiffer after 9 and 24 weeks, but at 1 and 5 weeks after renal artery clipping, no stiffening had occurred. With chronic exposure to high blood pressure, carotid arteries in this model stiffen, whereas in SHR-SP, pial arterioles become less stiff.

Vascular stiffening may involve changes in wall composition. An increased proportion of more distensible (smooth muscle and elastin) to less distensible (collagen and basement membrane) elements may underlie decreased pial arteriolar stiffness in SHR-SP. Vascular stiffness may also be modulated by adhesion molecules. Integrins, physical connectors between the extracellular matrix and cytoskeleton, also mediate signal transduction. They are composed of noncovalently linked α and β subunits, the interaction of which dictates ligand specificity. On the basis of the broad spectrum of integrin functions, vascular remodeling and/or stiffening probably involves changes in these anchorage sites. For example, fibronectin and its receptor, the α₅β₁ integrin, may modulate aortic stiffness in SHR.

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We hypothesized that age-related modifications in media components and/or integrins define resistance artery abnormalities in SHR. Specifically, we proposed that with aging in SHR, mesenteric resistance arteries stiffen in association with increased collagen deposition and integrin expression. To study the latter, we used \(^{125}\)I-echistatin, a 49–amino acid peptide found in venom of *Echis carinatus*, and its ability to bind \(\alpha_{v}\beta_{3}\) integrins and possibly other Arg-Gly-Asp (RGD)-binding integrins in a nondissociable manner.\(^9\) We also tested whether antihypertensive treatment with an AT\(_{1}\)-angiotensin receptor antagonist (irbesartan) or an angiotensin-converting enzyme inhibitor (fosinopril) would ameliorate hypertension-related differences in artery wall stiffness, media components, and integrin profile.

### Methods

**Animals**

The present study was conducted according to recommendations from the Animal Care Committee of the Clinical Research Institute of Montreal and the Canadian Council of Animal Care. Male SHR and Wistar-Kyoto (WKY) rats were obtained from Taconic Farms (Germantown, NY) and housed under a 12-hour light/dark cycle at 22°C and 60% humidity. Starting at 10 weeks, rats were fed 22°C and 60% humidity. Starting at 10 weeks, rats were fed starting at 10 weeks, rats were fed

**Preparation of Small Arteries**

Rats were killed at 6 or 20 weeks of age by decapitation, and mesenteric vasculature was isolated.\(^{10}\) A third-order artery (~2 mm) was placed on 2 glass microcannulas in a pressure myograph and adjusted so that vessel walls were parallel without stretch.\(^{11}\) Vessels were equilibrated (1 hour) under constant intraluminal pressure (45 mm Hg) with warm (37°C) physiological salt solution (PSS), which was bubbled with 95% air and 5% CO\(_2\) to achieve a pH of 7.4 to 7.45. Vessels were used if they constricted 50% in response to potassium (125 mmol/L KCl) with norepinephrine (10\(^{-4}\) mol/L) and to norepinephrine alone (10\(^{-5}\) mol/L). Endothelial integrity was confirmed if acetylcholine (10\(^{-3}\) mol/L) relaxed precontracted vessels >75%.

**Vascular Morphology**

Vessels were deactivated by perfusing with Ca\(^{2+}\)-free PSS containing 10 mmol/L EGTA for 30 minutes. Lumen and media dimensions were measured with the intraluminal pressure maintained at 45 mm Hg.

**Vascular Mechanics**

Intraluminal pressure was raised to 140 mm Hg 3 times, and arteries were unbuckled by adjusting the cannulas. Intraluminal pressure was increased stepwise\(^{10}\) to 140 mm Hg, and media and lumen dimensions were measured at 5 points. Initial diameter was measured at 3 mm Hg. If the vessel collapsed, lumen diameters at 10 to 140 mm Hg were fit to a third-order polynomial equation, and initial diameter was estimated.

**Morphological and Mechanical Formulas**

For definitions of parameters, see Reference 12. Media cross-sectional area is calculated as \((\pi/4)\times(D_e^2−D_i^2)\), where \(D_e\) and \(D_i\) are external and lumen diameters, respectively. Incremental distensibility is calculated as \((\partial P/\partial D_e)\times(D_e/D_i)\times100\) fractional change in lumen diameter \((\Delta D/D)\) per change in intraluminal pressure \((\Delta P)\). Cumulative strain \((\varepsilon)\) is \((D−D_i)/D_i\), where \(D\) is the lumen diameter for a given intraluminal pressure, and \(D_i\) is the original diameter at 3 mm Hg. Cumulative stress \((\sigma)\) is \((PD)/(2\pi R^2)\), where \(P\) is the intraluminal pressure (dyne/cm\(^2\)), and \(R\) and \(M\) are lumen diameter and media thickness, respectively. Elastic modulus was determined by fitting stress-strain data to \(\sigma=\sigma_c\varepsilon^{\nu}\), where \(\sigma_c\) was...
stress at $D_1$ and $\beta$ is a constant related to the rate of increase of the stress-strain curve. Tangential elastic modulus (ET) was calculated at several values of stress from the derivative of the aforementioned exponential curve: $ET=\sigma d\varepsilon = \beta \sigma e^\varepsilon$. Remodeling index is calculated as $100 \times \frac{(D_{1h}-D_{1m})(D_{1m}(D_{1h}))}{(D_{1h}-D_{1m})^2}$. Media/lumen ratio was greater, and media cross-sectional area of normotensive vessels and CSA, was the cross-sectional area of normotensive vessels. Growth index is calculated as $(CSA_n-CSA_h)/CSA_h$, where CSA, and CSA, are media cross-sectional areas of normotensive and hypertensive vessels, respectively.

### Determination of Resistance Artery Wall Composition
Deactivated arteries were pressurized at 45 mm Hg and processed for electron microscopy. Electron micrographs (final magnification ×12,000) of 12 to 15 sections per vessel were obtained with a JEM-1200EX electron microscope (JEOL Ltd) and scanned (ScanJet 4Ct, Hewlett-Packard), and areas occupied by smooth muscle cells, collagen, and elastin were measured by repeated tracing using Adobe Photoshop 3.0.

### Determination of RGD-Binding Integrins in Mesenteric Arteries of SHR and WKY Rats
Proteins were extracted from frozen mesenteric vasculature in lysis buffer containing (mmol/L) HEPES (pH 7.4) 50, NaCl 150, MgCl$_2$ 1.0, CaCl$_2$ 1.0, pepstatin 0.005, leupeptin 0.01, and phenylmethylsulfonylfluoride 1.0, along with Normet P-40 1% and aprotinin 50 KIU/mL. Proteins (10 μg) were incubated with $^{125}$I-echistatin (200,000 cpm), 5 mmol/L MnCl$_2$, and 50 mmol/L HEPES (pH 7.4) for 90 minutes at 25°C. Complexes were separated, without boiling and under nonreducing conditions, by 6% SDS-PAGE. Gels were dried (240°C), Band intensity was quantified by PhosphorImager (Molecular Dynamics) analysis.

### Identification of RGD-Binding Integrins
After electrophoresis, $^{125}$I-echistatin-integrins were transferred to a nylon membrane, which was immediately subjected to autoradiography for band localization. The membrane was blocked (0.05% Tween, 10% goat serum, 1% polyvinylpyrrolidone, and 2.5% to 5% milk), washed, and incubated with mouse anti-human integrin α1 (1:500, Chemicon International Inc), mouse anti-rat integrin β1 (1:1000, Pharmingen Canada), rabbit anti-human integrin α1 (1:2500, Chemicon International Inc), or hamster anti-rat integrin β1 (1:200, Pharmingen Canada). Membranes were washed in PBS and treated with the appropriate secondary antibody. Goat anti-mouse (1:10,000) and anti-rabbit (1:200,000) antibodies were conjugated with horseradish peroxidase. Membranes treated with goat anti-hamster antibody (1:1000) were exposed to streptavidin peroxidase conjugate (1:1000) for 30 minutes. Signals were detected by chemiluminescence.

### Data Analysis
Data are presented as mean±SEM. Unpaired Student $t$ test (young rats) and 1-way ANOVA followed by a Student-Newman-Keuls test (adult rats) (or Mann-Whitney test and Kruskal-Wallis tests, respectively, where standard deviations were different) and ANOVA for repeated measures were used as appropriate. Interaction means were analyzed for “simple main effects” by Student $t$ test for unpaired data (young rats) and Student-Newman-Keuls (adult rats) test. A value of $P<0.05$ was considered significant.

### Results
**Body Weight, Blood Pressure, and Small Artery Morphology**
Body weights were lower in SHR than in age-matched WKY rats, whereas in adult SHR, SBP was significantly higher than in age-matched WKY rats (Figure 1). Between 10 and 20 weeks of age, SBP rose significantly in untreated SHR but to a lesser degree ($P<0.01$) in SHR treated with irbesartan and fosinopril (Figure 1 and Table 2).

Lumen and external diameters were smaller (Figure 2), media/lumen ratio was greater, and media cross-sectional...
Figure 2. Lumen diameter–intraluminal pressure (top panels), external diameter–intraluminal pressure (middle panels), and media cross-sectional area–intraluminal pressure (bottom panels) curves in relaxed mesenteric arteries from 6-week-old (left) and 20-week-old (right) SHR and WKY rats (n=6). Error bars indicate SEM. *P<0.05 vs age-matched WKY rats. †P<0.05 vs age-matched, untreated SHR.
Figure 3. Incremental distensibility–intraluminal pressure (top panels), media stress–intraluminal pressure (middle panels), and media stress–strain (bottom panels) curves in relaxed mesenteric arteries from 6-week-old (left) and 20-week-old (right) SHR and WKY rats (n=6). Error bars indicate SEM. *P<0.05 and **P<0.01 vs age-matched WKY rats.
area was similar in vessels from SHR versus age-matched WKY rats (Table 2). In adult but not young SHR, media width was greater in SHR than in WKY vessels. Irbesartan reduced media width and media/lumen ratio, and fosinopril reduced media width and media cross-sectional area in adult SHR vessels (Tables 1 and 2).

In adult SHR, remodeling and growth indices were 88% and 24%, respectively (Figure 1), measured at an intraluminal pressure of 45 mm Hg. These represent percentages of the difference in lumen diameter between hypertensive and normotensive vessels that are attributable to eutrophic remodeling and to growth. Irbesartan and fosinopril produced hypotrophy resulting in growth indices of $-29\%$ and $-35\%$, respectively, relative to untreated SHR vessels.

**Vascular Mechanics**

Increasing intraluminal pressure to 140 mm Hg decreased the media/lumen ratio of relaxed vessels from young and adult SHR and WKY rats to similar degrees (data not shown) without altering media cross-sectional area, since the media is incompressible (Figure 2). Lumen and external diameters expanded less with increasing pressure in SHR versus WKY rats (Figure 2), indicating reduced capacity for passive dilation in mesenteric arteries from SHR irrespective of age. Distensibility was normal at physiological pressures (40 to 140 mm Hg, Figure 3) in SHR arteries, suggesting that when collagen has been recruited, distensibility is so low that media thickening does not lower it further.

Increasing intraluminal pressure increased media stress more in WKY than in SHR arteries ($P<0.05$), regardless of age (Figure 3). The stress-strain curve was shifted leftward in mesenteric arteries from SHR compared with those from age-matched WKY rats (Figure 3).

When plotted against intraluminal pressure or stress, incremental elastic modulus (wall stiffness) was similar between young WKY and SHR arteries (Figure 4, Table 1). In adult SHR, however, the slope of the incremental elastic modulus versus stress was augmented (Figure 4, Table 2). Pressure is transduced to the vessel wall as stress differentially depending on vessel geometry; thus, the relation between elastic

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**Figure 4.** Incremental elastic modulus–intraluminal pressure (top panels) and elastic modulus–media stress (bottom panels) curves in relaxed mesenteric arteries from 6-week-old (left) and 20-week-old (right) SHR and WKY rats (n=6). Error bars indicate SEM.
Vascular remodeling in hypertension involves 2 processes. In eutrophic remodeling, wall material appears rearranged around a reduced lumen without evidence of net growth. In hypertrophic remodeling, the media cross section is increased and encroaches on the lumen, indicating the presence of growth.3 As indicated by the growth index of 24%, vascular growth occurred in untreated 20-week-old SHR. The remodeling index of 88% may indicate eutrophic remodeling but is probably partially due to mechanical alterations, since wall components were stiffer in adult SHR.3

The interplay between structure and mechanics of resistance arteries from adult SHR is complicated. The thickened media, by lowering wall stress induced by high pressure, counters late-stage rigidity of wall components so that isobaric stiffness (resulting from wall components and vessel geometry) is not reduced in SHR. This is consistent with previous results obtained also in mesenteric resistance10 and carotid19 arteries. At 20 weeks of age, despite significant stiffening of wall components, geometric adaptation has occurred in SHR to maintain normal in vivo wall stress and stiffness, protecting the vessel wall from pressure-induced damage. In our initial report,10 isobaric stiffness of mesenteric arteries was also normal in adult SHR, but in that study, stiffness of wall components was lower, perhaps because of differences between groups of SHR. The important common finding is strict regulation of isobaric arterial stiffness in SHR, whether by modulating geometry or stiffness of wall components.

Stiffening of wall components in SHR agrees with our recent finding that collagen/elastin ratios are increased in 20-week-old SHR mesenteric small arteries versus WKY arteries,15 also confirmed here. Just as increases in elastin may have diminished stiffness of SHR-SP pial arterioles,6 increased collagen and, therefore, collagen recruitment at lower strain may increase stiffness of SHR mesenteric small arteries. Modifying media composition is not the sole mechanism involved in resistance artery stiffening in SHR. Irbesartan and fosinopril normalized the collagen/elastin ratio in SHR arteries, but only irbesartan reduced arterial stiffness. Also, in human subcutaneous resistance arteries, despite increased collagen/elastin ratios, wall stiffness was lower in vessels from mildly hypertensive patients.20

Changes in RGD-binding integrins that mediate adhesion of cells to the extracellular matrix may contribute to arterial remodeling and stiffness of the vascular wall. Integrins are abnormal in SHR, with a decrease in αβ integrins in young SHR arteries and an increase in αβ integrins in adult SHR. Increasing cell-matrix attachment sites, perhaps via fibronectin and α integrins, may participate in mechanical adaptation of aortas in SHR,8 where connections between smooth muscle cells and elastic lamellae are numerous.21 αβ and αβ integrins increased significantly in mesenteric arteries of SHR from 6 to 20 weeks of age. This may represent an increase in cell-matrix attachment sites intended to modulate arterial structure and mechanics in hypertension. However, as with collagen, irbesartan and fosinopril corrected integrin abnormalities in adult SHR arteries, but only irbesartan reduced arterial stiffness. Thus, neither wall composition nor integrin profile is the sole determinant of arterial stiffness. Perhaps topographical distribution (clustering?) of integrin-mediated attachment sites between ex-
tracellular matrix and vascular smooth muscle cells determines vascular stiffness in hypertension.

Likewise, expression levels of integrins are not sole determinants of eutrophic remodeling. Normalization of integrins by irbesartan and fosinopril was not paralleled by regression of remodeling, again suggesting that topographical distribution of attachment sites may be important. However, altered integrin status may be related to the growth component, because both irbesartan and fosinopril produced hypotrophic remodeling. Aside from acting as a physical joint, \( \alpha_\beta_3 \) integrins may promote growth functionally. Interactions between \( \alpha_\beta_3 \) integrins and tenascin-C (an extracellular matrix glycoprotein that is prominent in remodeling tissues) promote epidermal growth factor–dependent growth and survival of rat pulmonary artery smooth muscle cells. Because tenascin-C induction was also accelerated in SHR aortas, 23 Figure 6.
tenasin-C and/or other αβ3 integrin ligands may protect smooth muscle cells from apoptosis and promote proliferation and subsequent extracellular matrix deposition.

Vascular remodeling is preventable by early treatment with angiotensin-converting enzyme inhibitors and AT1-receptor antagonists. Losartan also improved vascular structure when administered late to SHR. In the present study, with mild blood pressure reduction (from 26 to 35 mm Hg), interrupting the renin-angiotensin system with irbesartan and fosinopril blunted small artery growth with growth indices of −29% and −35%, respectively, relative to untreated SHR. There was little effect on eutrophic remodeling.

Neither irbesartan nor fosinopril normalized blood pressure or remodeling completely. Vascular remodeling in SHR lowered wall stress (Figure 3), protecting the vessel from residually elevated blood pressure. If, in addition to preventing growth, irbesartan and fosinopril had reversed remodeling, high pressures would have increased wall stress significantly, predisposing the vascular wall to further damage. The remodeled lumen reduced circumferential wall tension and media stress, thereby acting protectively in the face of persistently high blood pressure after correction of the growth component.

In conclusion, SHR exhibit eutrophic remodeling of mesenteric resistance arteries by 6 weeks of age, and by 20 weeks, a combination of vascular growth and remodeling is seen. Interrupting the renin-angiotensin system interfered with growth but not eutrophic remodeling of resistance arteries in 20-week-old SHR. Whereas arterial stiffness was normal in young SHR, with aging to 20 weeks, components of the arterial wall became stiffer. In adult SHR, differences in lumen diameter may result from a combination of increased stiffness and eutrophic remodeling. A critical finding here is that the arterial wall adapts geometrically to tightly maintain physiologically relevant pressure-buffering capacity (ie, distensibility and isobaric stiffness) despite the presence of stiff components in the vessel wall. AT1-receptor blockade improved resistance artery wall stiffness. An increase in the proportion of less distensible (collagen) to more distensible (elastin) components in adult SHR, accompanied by an age-related increase in αβ3 and αβ1 integrins, may contribute to remodeling and stiffening of resistance arteries in this genetic model of hypertension.

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References


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