Effects of Protection From Pressure on Resistance Artery Morphology and Reactivity in Spontaneously Hypertensive and Wistar-Kyoto Rats

Stuart J. Bund, Kevin P. West, and Anthony M. Heagerty

The effects of regional hypotension on femoral resistance artery reactivity and morphology were investigated in spontaneously hypertensive rats (SHR) and control Wistar-Kyoto (WKY) rats. A partially constricting ligature (0.4 mm i.d.) was placed around the left external iliac artery at 5 weeks, which resulted in significantly reduced femoral mean arterial pressures distal to the ligature at 12 and 24 weeks. The femoral mean arterial pressure distal to the ligature in SHR was similar to that in WKY unprotected hind limbs. Resistance arteries (approximately 200 μm i.d.) were taken from unligatured and protected hind limbs and mounted in a myograph for reactivity and morphological measurements. Each experiment therefore utilized one artery distal to a ligature and one from the control hind limb. Histological examination revealed that nuclear density differed neither between strains nor between arteries from protected and unprotected femoral beds. Media thickness, media cross-sectional area, and media/lumen ratios were reduced in arteries from the hypotensive hind limb in SHR and WKY rats at 12 and 24 weeks. Arteries from the protected hind limbs of SHR were structurally indistinguishable from those from the normally perfused WKY vasculature. It is concluded that the medial content and maximal contractile responses of femoral resistance arteries from SHR and WKY rats are mainly determined by the local perfusion pressure and that normalization of perfusion pressure in SHR normalizes resistance artery structure. (Circulation Research 1991;68:1230–1240)

In all forms of hypertension, there appears to be a restructuring of the media in resistance arteries such that the muscle layers encroach on the lumen, thereby contributing to peripheral vascular resistance.1 However, the rate at which this proceeds and the magnitude of the response observed in the early phases of hypertension remain controversial. Indeed the nature of the histological change appears to vary; in spontaneously hypertensive rats (SHR), it is reported that there is hyperplasia (increased number) of the myocytes of resistance arteries,2–6 whereas in experimentally induced hypertension, hypertrophy (larger cells) occurs.7 There is evidence that the vascular smooth muscle cells of SHR are subject to greater intrinsic growth stimuli; vascular myocytes from SHR grow faster in culture than those from normotensive Wistar-Kyoto (WKY) control rats.8,9 This finding suggests that there is a genetically determined propensity to increased growth in SHR. Moreover, structural thickening of resistance arteries persists in SHR when blood pressure is lowered or maintained at normal levels,10–15 suggesting a possible increase in neural and/or humoral growth stimuli. Therefore, it is possible that the genotype of the cell might be abnormal in hypertension-prone humans and animals so that there is an abnormal growth response when tissues are exposed to pressor stimuli or to pressure-independent trophic factors. These two growth stimuli may not always be entirely mutually exclusive; experiments using cells grown in culture have demonstrated that vasoconstrictor agonists can induce cell multiplication16–18 or cell hypertrophy.19,20 One approach to examining this further is to observe the changes in vascular morphology in resistance arteries protected from the pressure rise seen as an animal ages. Previously, it has been shown that reduction of the perfusion pressure distal to a constriction in the vasculature results in a fall in resistance to blood
Animals

SHR and control WKY rats were obtained from the stock colony that is bred and held at Leicester University; they were maintained on standard rat chow and tap water ad libitum. Male rats were studied throughout. At 5 weeks of age, rats were either killed to provide femoral arteries for morphological measurements or were placed under ether anesthesia; the left external iliac artery of the anesthetized rats was exposed through a longitudinal incision in the abdominal wall. A stainless-steel wire with a length of 5 mm and a diameter of 0.4 mm was placed alongside the artery and tied firmly to the artery using a short length of 4-0 braided silk. In consequence, after removal of the wire, a loop of silk of fixed diameter remained around the artery; at this time, the ligature did not constrict the vessel. A similar procedure has been reported previously.\textsuperscript{23,25,26} The ligature site is indicated in Figure 1. Separate groups of both SHR and WKY rats underwent sham operations that involved the placement of a loose loop of silk approximately 1 cm in circumference around the artery; with this size of tie, there was no possibility of the blood vessel being compressed during the course of the experiment. Sham-operated rats were studied at 12 weeks. Indirect systolic blood pressure measurements were performed using the tail-cuff procedure under light ether anesthesia.

Drugs and Solutions

All experiments involving isolated resistance arteries were performed using physiological salt solution (PSS) of the following composition (mM): NaCl 119, KCl 4.7, CaCl\textsubscript{2} 2.5, MgSO\textsubscript{4} 1.17, NaHCO\textsubscript{3} 25, KH\textsubscript{2}PO\textsubscript{4} 1.18, EDTA 0.026, and glucose 5.5. Potassium PSS had the same composition as PSS but with an equimolar substitution of KCl for NaCl. All solutions were bubbled with 95\% O\textsubscript{2}–5\% CO\textsubscript{2} to give a pH of 7.4 at 37°C. (±)-Arterenol (norepinephrine [NE]) and cocaine were purchased from Sigma Chemical Co., Poole, UK.

Measurements of Vascular Morphology

At the ages of 5, 12, and 24 weeks, SHR and WKY rats were killed by stunning and cervical dislocation. Third-order branch vessels originating distal to the femoral artery from both left and right hind limbs were dissected out. The branching pattern was found to be consistent in either leg and between strains; consequently, all studies were performed on equivalent arteries. After dissection, arteries were cleaned of extraneous fat and connective tissue and mounted in a myograph\textsuperscript{24} that was modified to permit study of two vessels\textsuperscript{23} on two stainless-steel wires 40 µm in diameter, except vessels from rats at 5 weeks of age, which could only be mounted on 30-µm wire. Arteries were normally held relaxed in PSS. After mounting, the vessels were warmed to 37°C, and measurements were made of media thickness using water-immersion light microscopy while the vessels were held just under tension. Media cross-sectional area (equivalent to media volume per unit length) was calculated from the media thickness and internal circumference. The resting tension–internal circumference relation was determined for each segment of artery as previously described.\textsuperscript{24} From this the internal circumference (L\textsubscript{100}) the artery would have if relaxed and under a transmural pressure of 100 mm Hg (13.3 kPa) was calculated. The corresponding internal diameter (l\textsubscript{100}) was calculated as L\textsubscript{100}/π. Each artery was then set to the normalized effective internal diameter (l\textsubscript{c}), where l\textsubscript{c}=0.9 l\textsubscript{100}.\textsuperscript{24} In pilot studies, it was found that active tension developments were near maximal at this setting. Assuming constant media volume,\textsuperscript{28} the media thickness could be calculated for l\textsubscript{c}.

Vascular Reactivity

Arteries were normally held relaxed in PSS. After normalization to l\textsubscript{c}, arteries were activated twice with 10 µM NE in potassium PSS (NEK) and then with 10 µM NE in PSS, potassium PSS, and finally NEK. Activations were for 2 minutes, and the arteries were allowed to relax fully between each stimulation. The maximum tension development as measured on a polygraph (Grass Instrument Co., Quincy, Mass.) was produced with either the second or third application of NEK, and the greater response was taken as maximum for each artery. NE concentration–response relations were determined (0.02–10 µM, 2 minutes for each concentration) in the absence and presence of cocaine (3 µM), which was used to inhibit neuronal reuptake of NE.\textsuperscript{29} Cocaine was applied 10 minutes before and throughout the construction of the second concentration–response rela-
tion. Maximum responses during each 2-minute period were recorded for calculation purposes.

Vascular responses were calculated in three ways: 1) active tension, defined as force response divided by twice segment length; 2) active media stress, defined as active force divided by media thickness at l0; and 3) effective active pressure, calculated as two times active tension divided by l0 and therefore an estimate from Laplace’s equation for the pressure against which the vessel segment would be able to contract. These calculations have been described previously.24 Sensitivities to NE were determined in terms of pD2, where pD2 is the negative logarithm of the concentration required to produce half-maximal activation (i.e., pD2 = –log10 ED50). The effect of cocaine on the NE concentration–response curve was calculated from the shift in NE pD2 and calculated as NE pD2 – (NE+cocaine) pD2, such that a negative shift implies a leftward shift in the NE concentration–response relation.

**Histology**

After the reactivity studies, arteries were prepared for histological examination. Arteries were incubated in calcium-free PSS containing 0.1 mM EGTA at 37°C for 10 minutes to ensure complete relaxation. This solution was then replaced with prewarmed pre-fix solution (75 mM sodium cacodylate, 7% sucrose, and 2.5% glutaraldehyde). The myograph warming circuit was then switched off, and the arteries were left overnight. Next day, the arteries were carefully removed from the wire and stored in samples of the pre-fix solution. Arteries were then fixed in 2% glutaraldehyde, treated with osmium tetroxide, and embedded in epoxy resin. Transverse sections 0.5 μm thick were cut and stained with toluidine blue. At least five such sections were examined from each artery. Measurements of medial cross-sectional area were performed using a Videoplan image analysis system (Kontron Instruments Inc., Munich), and nuclear counts were performed at this time. Distorted areas in which nuclei could not be readily counted were not included. Nuclear density is expressed as nuclei per 104 μm².

**Measurement of Hind Limb Blood Pressure**

At 12 or 24 weeks, randomly selected rats from each strain underwent bilateral femoral artery cannulation to determine femoral mean arterial pressure (FMAP) in the control hind limb and also in the artery distal to the ligature. Cannulations were performed under ether anesthesia with PE-25 tubing (1.8 mm o.d., 0.4 mm i.d.), and pressures were recorded using a pressure transducer (Statham P23ID, Gould, Cleveland, Ohio) and chart recorder. Blood pressure recordings were made 4–4.5 hours after cannulation, while the rats were conscious and freely moving. A group of 5-week-old rats underwent left femoral artery cannulation using smaller (PE-10) tubing (0.61 mm o.d., 0.28 mm i.d.) but did not undergo the ligation procedure. FMAP was calculated from the following formula: FMAP = DP + 0.46 PP, where DP is diastolic pressure and PP is pulse pressure.

**Statistical Analyses**

Data from ligatured (protected) and unligatured (protected) hind limbs were compared within strains by Student’s paired t test, since measurements were made simultaneously and the effect of the ligature was of a priori interest. Between-strain comparisons were made using analysis of variance and the a posteriori Student-Newman-Keuls test where analysis of variance indicated a significant difference. Student’s paired t test was used to compare pD2 values in the presence and absence of cocaine to determine whether cocaine significantly influenced NE sensitivity in any vessel group. When data from both hind limbs could not be obtained, the data from the successfully cannulated artery or mounted vessel were normally omitted from the analysis. The exception was for the nuclear density measurements, where Student’s unpaired t test was performed because measurements could not be made for many arteries as a result of poor staining or media distortion leading to poor nuclear definition. Student’s unpaired t test was used to compare indirect systolic blood pressures, body weights, and 5-week rat data, since left and right hind limb data were calculated as mean values per rat. Significance was set at p < 0.05. Data are presented as mean ± SEM.

**Results**

**Five Weeks**

Indirect systolic blood pressures were not different between strains (Table 1). However, FMAP was significantly higher in SHR (Table 2) but may be a consequence of reduced tolerance of the WKY rats to the cannulation procedure since WKY rats displayed a much subdued postoperative behavior.

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**Table 1. Indirect Systolic Blood Pressures and Body Weights in Spontaneously Hypertensive and Wistar-Kyoto Rats**

<table>
<thead>
<tr>
<th>Age</th>
<th>Blood pressure (mm Hg)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHR</td>
<td>WKY</td>
</tr>
<tr>
<td>5 weeks</td>
<td>109±5 (16)</td>
<td>109±4 (16)</td>
</tr>
<tr>
<td>12 weeks</td>
<td>161±3* (22)</td>
<td>145±4 (19)</td>
</tr>
<tr>
<td>24 weeks</td>
<td>200±3† (16)</td>
<td>138±4 (19)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SHR, spontaneously hypertensive rats; WKY, control Wistar-Kyoto rats. Data do not include measurements of sham-operated rats. Values in parentheses indicate number of rats.

*p<0.002, †p<0.001, SHR vs. WKY.
There was no significant difference in any of the morphological measurements made on arteries at this age when comparing SHR to WKY rats (Tables 3 and 4).

Although it was possible to mount the small arteries taken from rats at 5 weeks of age and subsequently to make measurements of morphology, it was not possible to undertake pharmacological studies because of the development of spontaneous contractions in many of the arteries, poor tension maintenance, and poor relaxation between activations.

**Twelve Weeks**

**Femoral perfusion pressure.** FMAP was greater in unligatured hind limbs of SHR compared with WKY rats and was reduced by the ligation in both strains (Table 2). The FMAP in the ligatured limb of the SHR was similar to that observed in the normally perfused WKY hind limb (Table 2).

**Resistance artery structure.** Morphological measurements revealed that SHR femoral arteries normally display increased media thickness, media cross-sectional area, and media thickness/lumen diameter ratio (Figures 2–4). Normalized lumen diameters were not different between the two strains (Figure 5). Distal to a ligation, resistance arteries from both strains developed significantly less media thickness, reduced media cross-sectional area, and media/lumen ratio (Figures 2–4). The lumen diameter also increased in both rat strains but only reached statistical significance in WKY rats (Figure 5). The FMAP was similar in SHR ligatured and WKY unligatured hind limbs (Table 2), and the morphology of the arteries from the SHR protected bed and the WKY unprotected limb was indistinguishable (Figures 2–5).

**Vascular reactivity.** Sensitivity to NE was not altered by the application of the ligation in the WKY rat (Figure 6). In arteries from SHR hind limbs, there was reduced sensitivity to NE on the unligatured side; the protected side displayed a sensitivity to NE that was identical to that observed in the WKY arteries (Figure 6). Cocaine only enhanced the NE sensitivity in SHR unprotected arteries, but these arteries still remained less sensitive than those from WKY rats or protected arteries from SHR (Figure 6).

Active media stress was neither significantly different between strains nor significantly affected by the ligation (Figure 7, top panel). Therefore, the force-generating ability of the vascular smooth muscle was uninfluenced by strain or the ligation. Effective active pressures were higher in SHR and reduced by the ligation in both strains (Figure 7, middle panel). The ligation resulted in reduced maximum active tension values in each strain (Figure 7, bottom panel). However, despite the greater medial volume in arteries from the SHR unprotected hind limb in comparison with those from WKY unligatured limbs, these

### Table 2. Femoral Mean Arterial Pressures in Ligatured and Unligatured Hind Limbs of Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>SHR Unligatured</th>
<th>SHR Ligatured</th>
<th>WKY Unligatured</th>
<th>WKY Ligatured</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>94±3* (4)</td>
<td>. . .</td>
<td>67±3 (4)</td>
<td>. . .</td>
</tr>
<tr>
<td>12</td>
<td>145±7* (8)</td>
<td>96±7* (8)</td>
<td>93±4* (8)</td>
<td>62±4* (8)</td>
</tr>
<tr>
<td>24</td>
<td>165±6* (5)</td>
<td>125±4* (5)</td>
<td>125±4* (5)</td>
<td>91±6* (5)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SHR, spontaneously hypertensive rats; WKY, control Wistar-Kyoto rats. Values in parentheses indicate number of rats.

* p<0.01 vs. WKY unligatured hind limbs. t p<0.01 vs. unligatured hind limbs within a strain. tp=NS vs. SHR ligatured hind limbs.

### Table 3. Morphological Characteristics of Femoral Resistance Arteries From 5-Week-Old Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th></th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media thickness (μm)</td>
<td>7.87±0.38</td>
<td>7.13±0.36</td>
</tr>
<tr>
<td>Lumen diameter (μm)</td>
<td>96±5</td>
<td>102±4</td>
</tr>
<tr>
<td>Media/lumen ratio (x100)</td>
<td>8.65±0.53</td>
<td>7.53±0.59</td>
</tr>
<tr>
<td>Media cross-sectional area (μm²)</td>
<td>2,567±198</td>
<td>2,350±118</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=12 for all values. SHR, spontaneously hypertensive rats; WKY, control Wistar-Kyoto rats. Results are not significantly different for any parameter. Calculations are based on mean value per rat (one vessel from each hind limb).

### Table 4. Nuclear Densities in Femoral Resistance Arteries of Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>SHR Unligatured</th>
<th>SHR Ligatured</th>
<th>WKY Unligatured</th>
<th>WKY Ligatured</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>52.0±3.9 (9)</td>
<td>. . .</td>
<td>49.9±3.6 (11)</td>
<td>. . .</td>
</tr>
<tr>
<td>12</td>
<td>23.3±2.5 (13)</td>
<td>25.0±2.6 (11)</td>
<td>23.2±1.7 (13)</td>
<td>26.2±3.1 (6)</td>
</tr>
<tr>
<td>12 weeks*</td>
<td>18.7±3 (5)</td>
<td>22.7±5.1 (6)</td>
<td>23.1±1.9 (6)</td>
<td>21.0±1.8 (5)</td>
</tr>
<tr>
<td>24 weeks</td>
<td>16.7±1.8 (9)</td>
<td>18.8±1.6 (9)</td>
<td>15.1±0.9 (8)</td>
<td>18.7±1.8 (8)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SHR, spontaneously hypertensive rats; WKY, control Wistar-Kyoto rats. Values in parentheses indicate the number of rats. Units are nuclei per 10⁴ μm². Nuclear densities are not significantly different between strains and not significantly different between protected and unprotected femoral beds.

*Sham-operated rats.
arteries did not produce significantly greater maximum tension developments (Figure 7, bottom panel).

**Twenty-four Weeks**

**Femoral perfusion pressure.** The pattern of FMAP was similar to that seen at 12 weeks of age; on the unligatured side, hind limb pressures remained significantly higher in SHR compared with WKY rats, and in both strains the pressures in the unligatured side were higher than in the protected limb (Table 2). Again, in the SHR the FMAP on the ligatured side was not different from that observed on the unprotected side in the WKY rat (Table 2).

**Resistance artery structure.** Measurements of morphology also revealed a pattern similar to that seen at 12 weeks (Figures 2–5): media thickness, media/lumen ratio, and media cross-sectional areas were greater in

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**FIGURE 2.** Bar graph showing normalized media thickness of femoral resistance arteries. SHR, spontaneously hypertensive rats; WKY, control Wistar-Kyoto rats; n=15 and 12 for 12-week-old SHR and WKY, respectively; n=12 and 14 for 24-week-old SHR and WKY, respectively. Hatched bars indicate unligatured hind limbs; open bars indicate ligatured hind limbs. Error bars indicate ±SEM. *p<0.001 unligatured vs. ligatured hind limbs within rat strain. †p<0.05 SHR unligatured vs. WKY unligatured hind limbs at 12 weeks (p<0.01 at 24 weeks). SHR ligatured versus WKY unligatured hind limbs were not significantly different at either age.

**FIGURE 3.** Bar graph showing media cross-sectional areas of femoral resistance arteries. SHR, spontaneously hypertensive rats; WKY, control Wistar-Kyoto rats; n=15 and 12 for 12-week-old SHR and WKY, respectively; n=12 and 14 for 24-week-old SHR and WKY, respectively. Hatched bars indicate unligatured hind limbs; open bars indicate ligatured hind limbs. Error bars indicate ±SEM. *p<0.001 for 12-week-old SHR, p<0.01 for 12-week-old WKY, and p<0.05 for 24-week-old SHR unligatured vs. ligatured hind limbs within strain. †p<0.01 SHR unligatured vs. WKY unligatured hind limbs. SHR ligatured vs. WKY unligatured hind limbs were not significantly different at either age.

**FIGURE 4.** Bar graph showing the media/lumen ratio of femoral resistance arteries. SHR, spontaneously hypertensive rats; WKY, control Wistar-Kyoto rats; n=15 and 12 for 12-week-old SHR and WKY, respectively; n=12 and 14 for 24-week-old SHR and WKY, respectively. Hatched bars indicate unligatured hind limbs; open bars indicate ligatured hind limbs. Error bars indicate ±SEM. *p<0.001 for WKY and p<0.01 for SHR unligatured vs. ligatured hind limbs within strain. †p<0.01 for SHR unligatured vs. WKY unligatured hind limbs. SHR ligatured vs. WKY unligatured hind limbs were not significantly different.

**FIGURE 5.** Bar graph showing normalized lumen diameter in femoral resistance arteries. SHR, spontaneously hypertensive rats; WKY, control Wistar-Kyoto rats; n=15 and 12 for 12-week-old SHR and WKY, respectively; n=12 and 14 for 24-week-old SHR and WKY, respectively. Hatched bars indicate unligatured hind limbs; open bars indicate ligatured hind limbs. Error bars indicate ±SEM. *p<0.05 WKY unligatured vs. WKY ligatured hind limbs. SHR ligatured vs. WKY unligatured hind limbs were not significantly different.
unprotected arteries of SHR in comparison with WKY rats. Arteries protected from the developing pressure failed to display normal medial development in both strains. Again, at this age in the presence of similar perfusing pressures, the morphological parameters from vessels distal to the SHR ligature did not differ

FIGURE 6. Bar graphs showing norepinephrine (NE) sensitivity in the absence (top panel) and presence (middle panel) of cocaine. The bottom panel describes the cocaine shift: NE $pD_2$—NE (+cocaine) $pD_2$, where $pD_2$ is the negative logarithm of the concentration required to produce half-maximal activation. Error bars indicate $\pm$SEM. SHR, spontaneously hypertensive rats; WKY, control Wistar-Kyoto rats; n=12 or 13 SHR; n=9–12 WKY rats. Hatched bars indicate unligatured hind limbs; open bars indicate ligatured hind limbs. *p<0.05, **p<0.01 ligatured vs. unligatured hind limbs. †p<0.05, ††p<0.01 SHR unligatured vs. WKY unligatured hind limbs. §§Significant enhancement of NE sensitivity by cocaine at p<0.01. Cocaine shifts differ neither between strains nor between ligatured and unligatured hind limbs within strains.
from those observed in the unprotected WKY hind limb (Figures 2–5).

Vascular reactivity. In contrast to data from rats aged 12 weeks, resistance arteries from both protected and unprotected limbs were less sensitive to NE in SHR compared with WKY rats. The ligature had no effect on NE sensitivity in either strain (Figure 6). Cocaine did not significantly influence NE sensitivity, indicating that little NE reuptake occurs at this age.

At 24 weeks, effective active pressures were greater in SHR and reduced in both strains by the ligature (Figure 7, middle panel). The calculated media stress was neither significantly altered by the
ligature nor different between strains (Figure 7, upper panel). Active tension was attenuated in protected arteries only in WKY rats. SHR unprotected arteries did not produce greater active tension development when compared with WKY unprotected arteries (Figure 7, bottom panel).

Nuclear density. Nuclear density did not significantly differ between strains and was not altered by the ligature procedure (Table 4).

Sham-operated rats. Within strains, the loose ligation had no detectable effect on resistance artery morphology (Table 5) or reactivity. Therefore, there is no intrinsic difference between the arteries from left and right hind limbs. FMAP was not influenced by the sham procedure (data not shown).

Discussion
This experiment has investigated the effects of protecting the vasculature of one limb of a rat from the rise in blood pressure occurring as it ages; studies have been performed on normotensive and spontaneously hypertensive strains. We have used a myographic technique to measure reactivity and morphological changes in resistance arteries, and the protocol used has permitted the study of such vessels under standardized conditions.

Direct measurements of FMAP indicated that at 5 weeks of age the femoral bed was perfused at 40% higher pressure in SHR; however, the WKY rats appeared to tolerate the cannulation procedure less well, and in consequence, we believe that WKY FMAP was underestimated by this technique. Indeed, indirect systolic blood pressure measurements suggested no differences in blood pressure at 5 weeks of age, and morphology did not differ between the two strains at this age. This is in apparent contrast to the findings of Gray and DeMey,31 who reported that blood pressures in the SHR were raised from birth, and Nordborg and Johansson,32 who reported that blood pressures were significantly greater at 15 days and were associated with a general increase in arterial media thicknesses and media/lumen ratios. At 12 and 24 weeks, the FMAP on the unprotected side was significantly greater in SHR, and at both these time points, the FMAP was significantly attenuated by the partially constricting tie in both strains. Moreover, at both these ages, the protected hind limb of SHR had the same FMAP as that recorded in the unprotected WKY hind limb, therefore permitting a comparison of resistance artery morphology in animals of different genotype but under similar pressure. In previous studies,10-13,15 attenuation of the blood pressure rise in SHR by antihypertensive therapy has not necessarily resulted in a reduction of the cardiovascular hypertrophy normally found in the untreated SHR. However, it would appear that the selection of therapy may be important in this context: captopril11 and perindopril33 have been shown to attenuate the vascular changes, whereas hydralazine,10-12,33 pinacidil,13,34 verapamil,13 and bepridil13 do not. In addition, felodipine administration may fail to prevent the development of structural remodeling.35,36 Thus, the use of drugs has provided inconsistent evidence with regard to the relation between blood pressure and cardiovascular structural changes in SHR, possibly reflecting different influences of the drugs other than those mediated by the blood pressure reduction per se.

In the present study, we have demonstrated that by applying a ligature to an artery into which the animal grows, thereby gradually compressing the vessel wall and resulting in a normotensive vascular bed, the SHR resistance vasculature becomes structurally indistinct from unprotected WKY arteries; they assume “normal” medial geometry. At 24 weeks, the protected SHR arteries tended toward medial hypertrophy when compared with unprotected WKY arteries, suggesting that older SHR may be subject to greater neural and/or humoral influences on medial growth. However, the differences did not attain statistical significance. Therefore, it is suggested that SHR myocytes are not subject to significantly enhanced trophic stimuli, at least in the femoral resistance vasculature, except for the increased blood pressure itself. This is in contrast to what may be concluded from those reports10-15 in which the SHR vasculature developed as if exposed to high pressures despite attenuation of the blood pressure rise. The greater proliferation rate in culture of SHR myocytes in vitro8,9 is inconsistent with the apparent lack of an intrinsic genetic abnormality influencing myocyte growth directly in the SHR femoral resistance vasculature in vivo.

Table 5. Morphological Characteristics of Femoral Resistance Arteries of Sham-Operated Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th></th>
<th>SHR</th>
<th>WKY</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unligatured</td>
<td>Sham-ligated</td>
<td>Unligatured</td>
<td>Sham-ligated</td>
</tr>
<tr>
<td>Media thickness (µm)</td>
<td>12.74±0.68*</td>
<td>12.30±0.43</td>
<td>9.96±0.32</td>
<td>10.02±0.34</td>
</tr>
<tr>
<td>Lumen diameter (µm)</td>
<td>187±10</td>
<td>183±6</td>
<td>193±7.19</td>
<td>190±7</td>
</tr>
<tr>
<td>Media/lumen ratio (%)</td>
<td>7.48±1.12†</td>
<td>6.91±0.44</td>
<td>5.24±0.25</td>
<td>5.36±0.28</td>
</tr>
<tr>
<td>Media cross-sectional area (µm²)</td>
<td>7,847±464†</td>
<td>7,474±295</td>
<td>6,363±335</td>
<td>6,340±382</td>
</tr>
</tbody>
</table>

* p<0.01, † p<0.05 vs. WKY unligatured arteries.
The nuclear density measurements indicate that this parameter is not influenced by rat strain or partially constricting ligature. Therefore, when it is assumed that the myocytes were mononuclear and that the nuclear lengths and cell lengths were similar between strains and between ligatured and unligatured limbs, the media volume differences observed are a consequence of cell number differences. A further assumption is that the fraction of the media occupied by smooth muscle is similar between strains, as it is in the mesenteric circuit, and is not influenced by the ligature. Based on these assumptions, the data suggest that myocyte hyperplasia is present in the SHR femoral resistance vasculature as in the mesenteric circulation and that, distal to the ligature, cell numbers are reduced in SHR and WKY rats. In addition, the normalization of the media volume in protected SHR arteries is a consequence of attenuation of the hyperplasia. Obviously, further work needs to be done to test the validity of the assumption that unchanged nuclear density does indeed reflect unchanged cell size in this vascular bed.

It is possible that the ligature procedure might compromise sympathetic activity distal to the constriction, with a concomitant alteration in arterial growth response. Bevan and Lee have described how denervation may markedly influence arterial growth. However, the sympathetic supply to femoral resistance arteries is small, as indicated by fluorescence histochemistry and small shifts in NE sensitivity produced by cocaine application. In the present study, further evidence is provided for sparse innervation, since cocaine had only marginal effects on NE sensitivity, as has been previously observed. It is likely that the nerve density in these arteries was small, as has previously been noted, and that the silk tie has therefore had only minimal, if any, effects on nervous function. The cocaine shifts observed in this study are small compared with those of tail arteries (shift, -0.9) or mesenteric arteries (shift, -0.6 to -0.9), thus providing further evidence for sparse innervation in femoral resistance arteries. Consequently, it is unlikely that changes in sympathetic supply mediate significant differential influences on medial growth either between strains or between protected and unprotected beds. Therefore, it seems that the pressure to which a resistance artery is exposed is the major determinant of the medial structure, although neural or humoral influences cannot be discounted entirely.

Another consideration is the effect of flow on arterial structure. Flow reductions in one carotid artery of the rat and rabbit are associated with significant reductions in arterial lumen diameter, which appear to be endothelium dependent. If, indeed, changes in flow were the stimulus for arterial remodeling rather than a pressure drop distal to the constrictions in the carotid arteries (pressure measurements were not made), then the possibility exists for such a mechanism in the present study. However, the magnitude of the FMAP difference between protected and unprotected femoral arteries closely matches the order of the medial volume changes measured. In addition, it was our impression that there was no reduction in hind limb size distal to the ligature, although hind limb weights were not determined. Therefore, it is believed that flow restriction was not sufficient to inhibit tissue growth.

Arterial reactivity is the contractile response elicited by an agonist and is contributed to by the smooth muscle contractility, sensitivity, volume, and arrangement (i.e., how the myocytes are organized to determine the lumen diameter). Therefore, it is possible that changes in one component of reactivity might be compensated for by adjustment of another. In a recent report, the increased reactivity in prehypertensive SHR isolated perfused kidneys may have been at least partly compensated by a decreased NE sensitivity. We have also demonstrated that hypertension induced by chemical renal medullectomy is associated with a reduced NE sensitivity in mesenteric resistance arteries before significant medial growth occurs.

In the present experiment, there were no significant differences in media stress between femoral resistance arteries either between strains or between protected and unprotected arteries. Therefore, any differences in active tension or active pressure must be a consequence of altered arterial geometric design. Maximal active tension productions of unprotected SHR arteries were not significantly greater than those from WKY arteries, but the ligation procedure resulted in significant reductions in both strains at 12 weeks and in WKY rats at 24 weeks. It might appear at first sight that maximal contractile properties are not influenced as significantly as arterial structure by blood pressure differences within or between strains. However, effective active pressures are significantly greater in SHR and are reduced significantly by the ligature in both strains, resulting from the trend for smaller lumen diameters in unprotected arteries.

Although no sensitivity data were obtained for 5-week-old rats in this study, there is evidence from the 12-week-old rats that NE sensitivity is reduced in SHR femoral resistance arteries, possibly as a compensation for the medial hypertrophy. The depression of sensitivity was not an age-related intrinsic mechanism, since attenuation of the pressure rise distal to the ligature resulted in NE sensitivity comparable with that of WKY controls, in addition to attenuation of medial growth. The apparent compensation for increased medial content by reduced NE sensitivity was also observed at 24 weeks, but at this age blood pressure normalization was not associated with enhanced NE sensitivity in the SHR. There are conflicting data regarding NE sensitivity in the femoral vasculature of SHR and WKY rats. When isolated perfused hindquarters are used, there may be no difference or an increased sensitivity between SHR and WKY rats. An increased α₁ sensitivity is
observed until 14 weeks. Isolated femoral resistance arteries from SHR and WKY rats may also be equally sensitive. Pressure reduction by aortic constriction did not influence sensitivity, whereas using an iliac ligature local pressure reduction decreased NE sensitivity in SHR main femoral arteries compared with WKY femoral arteries. The disparities may reflect the different techniques used, the differing genetic backgrounds of the WKY controls, or age.

In summary, it has been demonstrated that the NE sensitivity of femoral resistance arteries is reduced in SHR compared with WKY arteries and that blood pressure normalization in the SHR can normalize NE sensitivity at 12 weeks. Femoral vascular smooth muscle force-generating ability is not different between SHR and WKY rats nor between low- and high-pressure beds. Maximal active tension developments are not tightly linked to blood pressure differences, but effective active pressures are, thus underlining the importance of arterial design rather than just medial content in the determination of contractile responses. These data provide evidence that SHR resistance arteries possess contractile properties similar to those of WKY rats when subjected to normal WKY blood pressures. Femoral resistance arteries from both SHR and WKY rats adjust structurally, depending on the pressure to which they are exposed, possibly by regulation of myocyte number. This experiment does not support the theory that SHR possess a load-independent propensity for increased vascular smooth muscle proliferation or growth, since resistance artery structure in a “normotensive” SHR hind limb is not significantly different from that measured in arteries from WKY rats exposed to the same blood pressure load.

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