Blood Flow Regulates the Development of Vascular Hypertrophy, Smooth Muscle Cell Proliferation, and Endothelial Cell Nitric Oxide Synthase in Hypertension

Hitoshi Ueno, Peter Kanellakis, Alex Agrotis, Alex Bobik

Abstract—Blood flow participates in vascular remodeling during development and growth by regulating cell apoptosis and proliferation. However, its significance in the development of vascular hypertrophy and vascular remodeling in hypertensive patients is not known. We investigated how changing blood flow through the common carotid artery (CA) of young adult rats rendered hypertensive via aortic coarctation affects CA hypertrophy and/or remodeling responses to hypertension. Blood flow was reduced by ∼50% as a result of ligation of the external CA immediately after hypertension was induced, and the effects of that procedure were compared with those in similarly treated normotensive rats. Reducing blood flow in the hypertensive animals markedly augmented the development of CA hypertrophy over the ensuing 14 days by increasing the vessel wall cross-sectional area. In those animals, CA lumen size was unaltered by reducing blood flow, as was CA structure in normotensive animals. The greater hypertrophy in the hypertensive animals with reduced blood flow was associated with enhanced smooth muscle cell (SMC) proliferation 3 days after the hemodynamic changes were induced. There also appeared to be more extensive remodeling of the endothelium in the hypertensive animals with normal flow; this was indicated by the greater frequency of apoptotic endothelial cells at that time. This reduction in blood flow also attenuated endothelial cell nitric oxide synthase expression in hypertensive animals but not in normotensive animals. Severe reductions in blood flow (∼90%) were required to reduce endothelial cell nitric oxide synthase in the normotensive animals. Increasing CA nitric oxide levels by perivascular application of S-nitroso-N-acetylpenicillamine (SNAP) to the CAs of hypertensive animals with reduced endothelial cell nitric oxide synthase attenuated the greater SMC proliferation. Thus, reduced blood flow in hypertensive animals promotes hypertrophy by enhancing SMC proliferation via mechanisms that reduce the inhibitory effects of nitric oxide on SMC proliferation. (Hypertension. 2000;36:89-96.)

Key Words: hypertension, experimental ■ blood flow ■ vascular hypertrophy ■ SNAP ■ apoptosis

Alterations in vascular structure are common in hypertension.1,2 In small arteries, the increase in vessel wall thickness/lumen (w/l) ratio contributes to greater vascular reactivity and elevated blood pressure,2 and in large arteries, a thicker wall is also a major risk factor for atherosclerosis.3,4 Two processes, vessel remodeling1,2 and/or vessel hypertrophy,1,2,6 are responsible for these changes in arterial structure. Vessel remodeling occurs through reorganization of apparently existing cellular and extracellular components around a smaller lumen.1 In contrast, hypertrophy involves vascular smooth muscle cell (SMC) proliferation, hypertrophy, and/or polyploidy,1,6 in addition to extracellular matrix deposition,7 which may be localized to the outer and/or inner regions of the wall or may occur throughout the vessel wall.1

The underlying mechanisms of vascular structural alterations are poorly understood. Vasoactive substances and hormones such as angiotensin II8 have been implicated, and studies in culture suggest that the mechanical deformation of endothelial cells (ECs) and SMCs is important.8,9 Pulsatile stretch of cultured SMCs or ECs stimulates their proliferation8-10 and growth factor production.8 Because blood flow can also change in hypertension,11 alterations in shear stress might also contribute to vascular structural changes. Continuous or pulsatile shear stress on cultured ECs modulates growth factor genes, including fibroblast growth factor-2 (FGF-2) and platelet-derived growth factor-B (PDGF-B).12 In cultured ECs nitric oxide synthase (eNOS) is also elevated by fluid shear stress,13 and its product (nitric oxide) can inhibit both SMC proliferation14 and migration.15

Recent studies16 in vivo indicate that reductions in shear stress induced by greatly reducing blood flow in the carotid artery (CA) of immature normotensive animals stimulates CA remodeling by altering EC and SMC apoptosis and proliferation rates. We investigated how more moderate reductions in blood flow affect CA structure in animals with developing hypertension. In the hypertensive animals, reductions in

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blood flow augmented the developing vessel wall hypertrophy without affecting lumen size (ie, there was no evidence of remodeling) via mechanisms that enhance SMC proliferation in the media and frequently in the intima. A reduction in nitric oxide production resulting from decreased eNOS expression appears responsible.

Methods

Animals and Experimental Design

Male Sprague-Dawley rats weighing 350 to 450 g from the Institute’s Biology Research Unit were used in this study. The animals were divided into 2 groups. The rats in one group were rendered hypertensive via aortic coarctation, and those in the other group underwent sham operation. In approximately one half of the animals in each group, the left external CA was ligated to reduce left common CA blood flow, and the remaining rats underwent sham operation. Blood pressure, blood flow, CA morphological factors, left ventricular weight (mg/body weight (g) ratio (LV/BW), indices of proliferation and apoptosis, and the expression of eNOS and FGF-2 were examined 3 and/or 14 days after surgery. The CAs of some hypertensive animals with reduced blood flow were treated with 3-nitroso-N-acetylpenicillamine (SNAP) applied perivascularly. In a subgroup of hypertensive and normotensive animals, the effects of severe reductions in blood flow (such as that produced by also ligating the internal CA) on eNOS were also examined. This study was approved by the Baker Institute Experimentation Committee.

Operations

Coarctation of the abdominal aorta was performed as previously described. The animals were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg IP), and a laparotomy was performed to expose the abdominal aorta. A silk ligature (size 3-0) was then tied around the aorta between the 2 renal arteries, and a standardized coarctation was produced by means of a stainless steel wire 0.4 mm in diameter. In the animals that underwent sham operation, the ligature was not tied. Carotid artery blood flow in half the sham-operated (normotensive) animals and in those with aortic coarctation (hypertensive animals) was reduced during surgery by ligation of the internal CA. Blood pressure, blood flow, CA morphological factors, left ventricular weight (mg/body weight (g) ratio (LV/BW), indices of proliferation and apoptosis, and the expression of eNOS and FGF-2 were examined 3 and/or 14 days after surgery. The CAs of some hypertensive animals with reduced blood flow were treated with 3-nitroso-N-acetylpenicillamine (SNAP) applied perivascularly. In a subgroup of hypertensive and normotensive animals, the effects of severe reductions in blood flow (such as that produced by also ligating the internal CA) on eNOS were also examined. This study was approved by the Baker Institute Experimentation Committee.

CA Blood Pressure and Blood Flow

Intra-arterial systolic blood pressure (SBP) was measured via a catheter inserted into the right external CA in rats that had been anesthetized with ethyl carbonate (urethane, 1.5 g/kg IP). In some animals, the right femoral artery SBP was also measured in a similar manner. SBP was recorded for 30 minutes by means of a Grass Recorder (Grass Instruments) and was averaged. The left common CA blood flow was measured for 30 minutes by means of an ultrasonic Doppler flow probe (Transonic Flow probe 2SB1167, Transonic Systems, Inc) connected to a transit-Time-Flow-Meter (Transonic Systems, Inc), and the readings were averaged.

Vessel Collection and Processing

CAs were isolated and were processed as described previously. The rats were deeply anesthetized with sodium pentobarbitone (100 mg/kg body wt), and the vessels were perfused fixed in situ with 4% paraformaldehyde in 0.1 mol/L sodium phosphate buffer (PBS, pH 7.4) at the animal’s SBP reading. The CAs were dissected into proximal and distal sections, were postfixed in osmium tetroxide, and were embedded in Epon. Arteries for immunohistochemical study were perfused with saline, mounted in optimal cutting temperature compound (OCT) (Tissue Tek), and stored at −80°C.

Vessel Morphometry

CA morphologic characteristics were assessed by means of Epon-embedded sections 1 μm thick that were stained with 1% toluidine blue in 1% borax. The media was defined as the region between the internal and external elastic laminae, the intima area was defined as the region between the endothelium and the internal elastic laminae, and the lumen area as the area encompassed by the endothelium. The morphometric result for each vessel is the average of measurements from its proximal and distal regions.

Immunohistochemistry and Cell Proliferation

FGF-2 and eNOS expression was assessed by immunohistochemistry. Briefly, 6 μm cross sections of arteries were postfixed in 4% paraformaldehyde (pH 7.4), were treated with 3% H2O2 in PBS, and were incubated with 10% horse serum in 0.1 mol/L PBS (pH 7.4) before application of the primary antibody, which was either an anti-FGF-2 antibody [anti-human monoclonal antibody (1:1000), du Pont Nenours and Company, (#DE6)] or an anti-eNOS antibody [mouse anti-human monoclonal antibody (1:1000), (Transduction Laboratories) (#N30020)]. After having been washed, the sections were incubated with the biotinylated anti-mouse IgG [Zymed Laboratories, (#61-1640) or a biotinylated anti-goat IgG (Vector Laboratories Inc) (#BA-2001)] and both of which were diluted 1:200. Antigenic peptides were detected by means of the avidin-biotin-peroxidase complex method (Vector Laboratories Inc) with 3,3’-diaminobenzidine tetrachloride as the chromogen. Sections were counter-stained with hematoxylin. A semiquantitative assessment of relative intensity of expression of the immunoreactive peptides was carried out as described by Ishikawa et al. The system of graded intensities23 from 0 to 4+ was used; the symbol – represented no staining; 1-, weak staining; 2+, moderate staining; 3+, intense staining; and 4+, very intense staining. The numbers of proliferating ECs and SMCs were estimated from the percentage of cells expressing proliferating cell nuclear antigen (PCNA), which was detected with a mouse monoclonal anti-PCNA (1:200) clone PC-10 [Santa Cruz Biotechnology, Inc., #sc-56]; a mouse IgG1 was used as a control. Positive cells were detected by means of a biotinylated anti-mouse IgG (1:200) and the avidin-biotin-peroxidase complex method. The cells were counterstained with hematoxylin. No fewer than 300 nuclei of SMCs and 100 nuclei of ECs were counted.

TUNEL-Positive Cells

Terminal deoxynucleotidyl transferase mediated dUTP-X nick end labeling (TUNEL) of cells was performed on 6 μm cross-sections postfixed in 4% paraformaldehyde (pH 7.4). The sections were briefly irradiated with microwaves and were placed in 0.1 mol/L Tris-HCl containing 3% BSA and 20% normal bovine serum (pH 7.5). Terminal deoxynucleotidyl transferase enzyme and dUTP-X were used according to the manufacturer’s specifications (in situ death detection kit, #1684817, Boehringer-Mannheim). Annexin V binding and labeled DNA nicks were detected by means of a peroxidase-coupled monoclonal antibody against fluorescein (converter-POD, #1684817, Boehringer-Mannheim), 3,3’-diaminobenzidine, and H2O2. Nuclei were counterstained as before, and TUNEL-positive cells were counted.

Statistical Analysis

Data are presented as mean±SEM. Differences between groups were analyzed via 1-way ANOVA after testing for normality by means of the Kolmogorov-Smirnov test (SigmaStat, Jandel Scientific); the Newman Keuls test was used for post hoc analyses. Data failing the normality test were analyzed by nonparametric analysis of variance, and where differences were detected, the Mann-Whitney rank sum test was used to determine significance. The expression of eNOS, and FGF-2 was also analyzed in this manner. P<0.05 was considered significant.

Results

BW and Hemodynamics

Three days after the operations had been performed, CA SBP in the rats with aortic coarctation was ≈63 mm Hg higher.
than that in the normotensive rats that underwent sham operation (P<0.05; Table 1). At that time, the differences in SBP across the coarctation in the hypertensive animals, which were reflected by differences in pressure between the carotid and femoral arteries, were 60 mm Hg in the hypertensive normal blood flow animals and the hypertensive reduced blood flow animals (n=3 per group). There were no significant differences in SBPs in the animals with normal or reduced left CA blood flow (P>0.05; Table 1), and there was also no difference in heart rate (P>0.05, Table 1). LV/BW ratios were increased in the hypertensive animals 9% to 18% (P<0.05; Table 1). LV/BW in the hypertensive reduced blood flow animals was 8% greater than that in the hypertensive normal blood flow group (P<0.05). Left CA blood flow in normotensive animals with ligated external CAs was reduced by >50%, and in hypertensive animals it was reduced by ≈40% (both, P<0.05). There was no difference in CA blood flow between the normotensive normal blood flow group and the hypertensive normal blood flow group (P>0.05; Table 1). Two weeks after the sham aortic operation, SBP was 20 to 30 mm Hg higher than it was 3 days after the operation (P<0.05), but LV/BW ratios and heart rates were unaltered (Table 1). SBPs in the hypertensive animals were identical to those 3 days after the operation, and increases in LV/BW ratios were small (Table 1). CA blood flow increased slightly during the 2 weeks after the external CA was ligated in the normotensive animals, but in hypertensive reduced blood flow animals, there was a small decrease in CA blood flow (Table 1).

**CA Structure**

The left CA cross-sectional wall area (CAWA; ie, media plus intima) of the normotensive normal blood flow animals and the hypertensive normal blood flow group (P>0.05); the CAWA was also unaffected when blood flow was reduced for 14 days (P>0.10; Figure 1). Three days after hypertension was induced, the CAWA was ≈9% higher than in normotensive normal blood flow animals, but the difference was not significant (P>0.05). After 14 days of hypertension in the hypertensive normal blood flow group, the CAWA was increased by 45% (normotensive normal blood flow group, P<0.05). In the hypertensive reduced blood flow group, the increase in CAWA 3 days after the CA blood flow was reduced averaged 39% (normotensive normal blood flow group, P<0.05) and was similar to that of the CAWA observed in the hypertensive normal blood flow group in which the animals were hypertensive for 14 days (39% versus 45%; P>0.05; Figure 1). The increases in CAWA were also time dependent in the hypertensive reduced blood flow group (Figure 1), and after 14 days, the CAWAs were 80% greater than in normotensive normal blood flow group animals (P<0.05). In addition, in one third of the left common CAs, a significant smooth muscle–rich intima developed (Figure 2), which was not observed in any of the hypertensive normal blood flow animals or the normotensive reduced blood flow animals (Figure 2). Neither the reduction in flow nor its combination with hypertension significantly affected CA flow.

**Figure 1.** Effects of reduced blood flow and hypertension on carotid artery wall area (media plus intima) and lumen cross-sectional areas in rats 3 days (open bars) and 14 days (shaded bars) after induction of the hemodynamic changes. Results are the mean±SEM; number of animals per group are in parentheses. *Normotensive normal blood flow animals, P<0.05; 13 days after induction of hemodynamic changes in hypertensive reduced blood flow animals, P<0.05.
lumen size ($P>0.10$, Figure 1). Thus hypertrophy of the CA wall is the only contributor to the increase in calculated CAWA/lumen cross-sectional area (Wa/La) ratio, which averaged 0.36 and 0.28 in hypertensive reduced blood flow animals and hypertensive normal blood flow animals, respectively. The Wa/La was similar in the normotensive animals and averaged 0.22 and 0.22 in normotensive normal blood flow animals and normotensive reduced blood flow animals, 14 days after operation.

**Vascular Cell Proliferation and Apoptosis**

Because changes in vessel structure in normotensive and hypertensive animals can involve cell proliferation and/or apoptosis, we investigated how reductions in blood flow during the development of hypertension affected those processes. Reductions in CA blood flow did not significantly affect the frequency of PCNA positive ECs in normotensive animals ($P>0.05$; Figure 3). However, PCNA frequency in ECs was increased by hypertension, and 3 days after operation averaged nearly 8% in the hypertensive groups. PCNA was barely detectable in the ECs of the normotensive normal blood flow group ($P<0.05$; Figures 3 and 4). After 2 weeks of hypertension, PCNA frequency in ECs was reduced by $\approx 50\%$ (Figure 3). Three days of hypertension also increased the frequency of TUNEL positive ECs in the left CA (Figure 4); this was greatest in the hypertensive normal blood flow group, which averaged 19% ($P<0.05$; Figure 3). Thereafter, the frequency of TUNEL-positive ECs decreased below 5% (Figure 3), despite continuing high blood pressure (Table 1). In the normotensive normal blood flow groups, <2% of the ECs were TUNEL positive, and an apparent increase in TUNEL-positive ECs in the normotensive reduced blood flow animals was not significant (from normotensive reduced blood flow animals, $P>0.05$).

Reductions in blood flow in hypertensive animals markedly increased the frequency of PCNA-positive SMCs in the CAs and averaged 19% in hypertensive reduced blood flow animals 3 days after the hemodynamic changes were induced ($P<0.05$; Figure 4). At 2 weeks after operation, the frequency of PCNA positive SMCs in the hypertensive animals was similar to that in normotensive normal blood flow animals ($\approx 1\%$) (Figure 3). Reducing blood flow in the normotensive animals for 3 days caused a very small and not significant increase in PCNA-positive cells ($P>0.05$; Figure 3). The frequency of TUNEL-positive SMCs in the CA of hypertensive animals was below 5%, as were any small flow-induced changes (Figure 4). Thus SMC proliferation is a major contributor to greater CA hypertrophy when blood flow is reduced in those with hypertension.

**Figure 2.** Photomicrographs depicting the increases in carotid artery wall area of hypertensive animals with normal or reduced blood flow compared with that in normotensive animals with normal or reduced blood flow 14 days after hemodynamic changes were induced. M indicates media; I, intima; and A, adventitia. Magnification $\times 328$. **Figure 3.** Effects of reduced blood flow and/or hypertension on endothelial cell (left) and SMC (right) proliferation and apoptosis in the carotid artery. PCNA expression was used to assess proliferation and TUNEL was used to assess apoptosis frequency 3 days (open bars) and 14 days (shaded bars) after hemodynamic changes were induced. Results are means $\pm$ SEM; animal numbers per group are in parentheses. *$P<0.05$ from normotensive normal blood flow animals.
Effect of Flow and Hypertension on eNOS Expression

Because eNOS is increased by angiotensin II and because it can be regulated by both pressure and shear stress and its product, nitric oxide, inhibits SMC proliferation and migration, we also examined how the expression of eNOS in the CA was altered by flow during hypertension. In normotensive animals, eNOS was unaffected by a 3-day reduction in blood flow; rather, reductions were observed only when blood flow was very low (90% reduction, Table 2 and Figure 5, left). Expression was highest in hypertensive normal blood flow animals, in which eNOS was 50% greater than in the normotensive normal blood flow group (Table 2; Figure 5, left). When blood flow was reduced by 50% in hypertensive animals, eNOS levels were, on average, approximately one half of those in hypertensive normal blood flow animals (for difference, \( P < 0.05 \); Table 2 and Figure 5, left). The eNOS in those animals also tended to be lower than in corresponding normotensive animals. Thus moderate reductions in blood flow attenuate eNOS expression in hypertensive animals but not in normotensive animals and could contribute to the flow-mediated increase in SMC proliferation.

Vessel NO Increases and SMC Proliferation in Hypertension

To determine whether increasing NO levels in CAs of hypertensive reduced blood flow animals attenuates SMC proliferation, the NO donor SNAP was also applied at surgery to the CA, and proliferation was assessed 24 hours later. In CAs treated with SNAP, only 13.6 ± 2.3% of the SMCs were PCNA positive, compared with PCNA positivity of 30.6 ± 2.6% in controls (\( P < 0.05 \); Figure 6). SBP was unaffected by SNAP and averaged in those 2 groups 149 ± 8 and 146 ± 7 mm Hg, respectively (\( P > 0.05 \)).

Fibroblast Growth Factor-2 Expression

Because FGF-2 has been associated with increased eNOS expression and can induce SMC proliferation in vivo, we compared its expression in the CA of the normotensive and hypertensive animals 3 days after hemodynamic changes were induced. FGF-2 was present in CA ECs of the normotensive normal blood flow animals, but in the normotensive reduced blood flow animals, it was mostly absent (Table 2, Figure 5, right). High amounts of FGF-2 were present in the ECs of hypertensive normal blood flow animals, substantially more so than in ECs of the hypertensive reduced blood flow group (\( P < 0.05 \)). FGF-2 in CA media of hypertensive and normotensive animals was similar (Table 2, Figure 5, right).

Discussion

Complex responses of ECs and SMCs to vasoactive agents and blood pressure are major determinants of vascular hypertrophy in hypertension. We have demonstrated that blood flow also regulates vascular hypertrophy. A moderate reduction in CA blood flow during the development of hypertension further increases CA hypertrophy without inducing vessel remodeling by altering the pattern of SMC and EC proliferation.

TABLE 2. Expression of Immunoreactive Endothelial Cell Nitric Oxide Synthase and FGF-2 in the Left Carotid Artery of Hypertensive and Normotensive Animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Endothelial Cell Nitric Oxide Synthase</th>
<th>FGF-2</th>
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<tr>
<td></td>
<td>Endothelium</td>
<td>Media</td>
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<td>Normotensive animals</td>
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<tr>
<td>NT-NF</td>
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<tr>
<td>NT-LF</td>
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<td>HT-NF</td>
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Results show the extent of positive immunoreactivity: – indicates negative; +, weak staining; ++, moderate staining; ++++, intense staining; and +++++, very intense staining. Results are the average of 5 animals in each group. NT indicates normotensive; NF, normal blood flow; RF, reduced blood flow; LF, low blood flow; and HT, hypertensive.

\*P < 0.05 compared with NT-NF, †P < 0.05 compared with NT-RF, ‡P < 0.05 compared with HT-NF, #P < 0.05 compared with NT-RF.
proliferation and apoptosis. The greater hypertrophy is primarily due to greater SMC proliferation, which is probably a consequence of removal of NO inhibitory effects on SMC proliferation and migration. In the hypertensive animals, blood flow reductions did not alter FGF-2, but rather decreased the expression of eNOS, the product of which (NO) can prevent SMC proliferation\(^{14}\) and migration.\(^{15}\) In contrast, in normotensive animals, similar reductions in flow affected neither eNOS expression nor CA structure.

Fluid shear stress and circumferential stretch contribute to normal vessel geometry and can contribute to the pathological changes in vascular structure associated with hypertension and atherosclerosis.\(^7\) Short-term cyclic stretch in vitro induces ECs to proliferate\(^{10}\) and to produce platelet-derived growth factor-B (PDGF-B).\(^{12}\) and shear stress on cultured ECs stimulates FGF-2\(^{13}\) and eNOS production.\(^{14}\) Our studies indicate that an increase in the circumferential stretch of ECs also appears important for their proliferation in vivo during the development of vascular hypertrophy. Early in the development of CA hypertrophy, PCNA was expressed in \(\approx 8\%\) of the ECs, but was barely detectable in the ECs of normotensive animals; it did not appear to be influenced by the reduction in blood flow. Because vessel lumen size was not altered by either hypertension or the reduction in blood flow, the increase in endothelial cell PCNA expression in the hypertensive vessels probably reflects a modification of endothelial structure associated with developing vessel hypertrophy. The finding of an increase in TUNEL-positive ECs during the development of vascular hypertrophy suggests a process of endothelial remodeling that also involves the loss of ECs as a result of apoptosis. Recently, a cycle involving cell proliferation-migration-loss has been reported during remodeling of EC monolayers in vitro.\(^{30}\) At present, one can only speculate about the mechanism responsible for the higher apoptosis of ECs in the vessels undergoing hypertrophy. Oxidant stress can be elevated in those with hypertension,\(^{31}\) and it is possible that hydrogen peroxide, a potent stimulant of EC apoptosis, is involved.\(^{32}\)

Our findings on eNOS in ECs of the vessels undergoing hypertrophy suggest an active involvement of the endothelium in regulating the development of vascular hypertrophy in hypertensive animals. Nitric oxide limits mitogenic responses of cultured SMC\(^{14}\) and also their in vitro migratory ability.\(^{15}\) Our finding that a moderate reduction in blood flow in the
hypertensive animals markedly reduces eNOS and augments vascular hypertrophy is consistent with the theory of a growth inhibitory role of NO in hypertension. The observed reduction in eNOS may also account for the development of the SMC-rich intima in many of the hypertrophied CAs of the hypertensive reduced blood flow animals, which is an effect dependent on SMC migration from the media to the intima. The lack of any similar flow-dependent reductions in eNOS in CA of normotensive animals is consistent with these mechanisms and suggests that the endothelium of hypertensive animals is more responsive to changes in blood flow.

Smooth muscle cell proliferation is a major mechanism responsible for the greater CA hypertrophy in the hypertensive animals with reduced blood flow, because apoptosis of SMCs, although apparent, was not substantially affected; in adult spontaneously hypertensive rats, a resistance of SMCs to apoptosis has been implicated in vascular hypertrophy. The lack of any similar flow-dependent reductions in eNOS in CA of normotensive animals is consistent with these mechanisms and suggests that the endothelium of hypertensive animals is more responsive to changes in blood flow. Plasma renin activity is increased in animals with reduced blood flow, because apoptosis of SMCs, although apparent, was not substantially affected; in adult spontaneously hypertensive rats, a resistance of SMCs to apoptosis has been implicated in vascular hypertrophy. The observed increases in SMC proliferation in the CA of the hypertensive animals is consistent with the contribution of circulating angiotensin II to vascular hypertrophy. Plasma renin activity is increased in animals with aortic coarctations and their vascular hypertrophy can be prevented with an angiotensin II receptor antagonist. Also, angiotensin II stimulates SMC proliferation in CAs. Although angiotensin II-induced SMC proliferation in vivo appears dependent on FGF-2, and in culture angiotensin II elevates FGF-2 expression in SMCs, we did not observe any FGF-2 elevation in the SMCs of the hypertensive animals. Whether increased release of FGF-2 from cells in the CA or an increase in another growth factor stimulated by angiotensin II accounts for SMC mitogenesis in hypertensive animals remains to be determined.

In summary, our study has demonstrated that in young adult hypertensive animals, moderate reductions in blood flow (less than those reported to induce vessel remodeling in normotensive animals) further augments vascular hypertrophy by elevating SMC proliferation. This effect appears to be due to withdrawal of the inhibitory effects of nitric oxide on SMC proliferation and migration during the development of vessel hypertrophy, a consequence of reduced eNOS expression. Such a mechanism may contribute to the development of a significant SMC-rich intima in many hypertrophied CAs, which in humans is a major risk factor for stroke.

References


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