The role of the renin-angiotensin system and oxidative stress in spontaneously hypertensive rat mesenteric collateral growth impairment

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Submitted 27 November 2006; accepted in final form 29 January 2007

The role of the renin-angiotensin system and oxidative stress in spontaneously hypertensive rat mesenteric collateral growth impairment. Am J Physiol Heart Circ Physiol 292: H2523–H2531, 2007. First published February 2, 2007; doi:10.1152/ajpheart.01296.2006.—Recent clinical and animal studies have shown that collateral artery growth is impaired in the presence of vascular risk factors, including hypertension. Available evidence suggests that angiotensin-converting enzyme inhibitors (ACEI) promote collateral growth in both hypertensive humans and animals; however, the specific mechanisms are not established. This study evaluated the hypothesis that collateral growth impairment in hypertension is mediated by excess superoxide produced by NAD(P)H oxidase in response to stimulation of the ANG II type 1 receptor. After ileal artery ligation, mesenteric collateral growth did not occur in untreated, young, spontaneously hypertensive rats. Significant luminal expansion occurred in collaterals of spontaneously hypertensive rats treated with the superoxide dismutase mimetic tempol, the NAD(P)H oxidase inhibitor apocynin, and the ACEI captopril, but not ANG II type 1 (losartan) or type 2 (PD-123319) receptor blockers. The ACEI enalapril produced equivalent reduction of arterial pressure as captopril but did not promote luminal expansion. This suggests the effects of captopril on collateral growth might result from its antioxidant properties. RT-PCR demonstrated that ANG II type 1 receptor and angiotensinogen expression was reduced in collaterals of untreated rats. This local suppression of the renin angiotensin system provides a potential explanation for the lack of effect of enalapril and losartan on collateral growth. The results demonstrate the capability of antioxidant therapies, including captopril, to reverse impaired collateral artery growth and the novel finding that components of the local renin angiotensin system are naturally suppressed in collaterals.

collateral growth; arteriogenesis; antioxidant; angiotensin-converting enzyme inhibitor; hypertension; captopril

NATURAL COMPENSATION FOR PERIPHERAL arterial occlusive disease includes both the formation and growth of new blood vessels in ischemic tissues and the flow-mediated enlargement of preexisting bypass vessels. This latter process has been termed collateral growth, arteriogenesis, or collaterogenesis. Strong evidence supports a role for angiotensin II (ANG II) signaling through the ANG II type 1 receptor (AT1R) in both promotion and inhibition of angiogenesis (24). Few studies have investigated the role of the renin-angiotensin system (RAS) or ANG II in collateral growth, even though collateral growth provides greater hemodynamic benefit for peripheral arterial insufficiency than capillary formation or angiogenesis (62, 69). Recent clinical and animal studies have shown that collateral growth is impaired in the presence of risk factors for vascular disease, including hypertension. Angiotensin-converting enzyme inhibitors (ACEI) have been shown to promote collateral growth in both hypertensive humans (35) and animals (17). The specific mechanisms responsible for the impairment in hypertension and its reversal by ACEI have not been established. Given the common use of ACEI and ANG II receptor blockers (ARB) for treatment of vascular disease, including peripheral arterial disease, it is of critical importance to elucidate the mechanisms by which these drugs impact the physiological response to arterial occlusion.

We selected the spontaneously hypertensive rat (SHR) to investigate the mechanisms by which ACEI are able to reverse collateral growth impairment. In this animal model, vascular adaptations to arterial occlusion have been shown by two independent groups to be impaired in different organ systems: the hindlimb (16, 17) and mesentery (59). In the hindlimb, chronic administration of the ACEI ramipril increases tissue perfusion without altering skeletal muscle capillary or arteriole density (17). These authors speculated that the improved perfusion resulted from enhanced collateral function.

The SHR has several characteristics similar to human hypertension that may be involved in collateral growth impairment and that may be corrected by manipulation of RAS. These characteristics include endothelial dysfunction and elevated levels of oxidative stress and ANG II. Endothelial dysfunction or reduced nitric oxide (NO) bioavailability may have a primary role in the impairment of collateral growth. Several groups have shown that, during collateral growth or shear-mediated outward arterial remodeling, endothelial nitric oxide synthase (eNOS) and NO are essential for altered gene expression/activity, matrix metalloproteinase activation, and cell recruitment (9, 34, 56, 57, 71). Excessive oxidative stress can produce endothelial dysfunction and eNOS uncoupling. The male SHR has elevated vascular oxidant production throughout the vasculature (10, 53, 72). NAD(P)H oxidase is a major, if not the predominant, source of elevated superoxide in the SHR vascular wall (22, 60). ANG II activates NAD(P)H oxidase activity via the AT1R (29) and expression of AT1R and ANG II type 2 receptor (AT2R) is elevated in young male SHR (40, 50).

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Based on these reported findings, we hypothesized that SHR collateral growth impairment is pressure independent and mediated by excess superoxide produced by NAD(P)H oxidase in response to stimulation of AT1R by ANG II. When initial results indicated that SHR collateral growth was enhanced by the ACEI captopril, the superoxide dismutase mimetic tempol, and the NAD(P)H oxidase inhibitor apocynin, but not by the AT1R antagonist losartan, additional studies were designed to clarify the mechanisms by which captopril had a beneficial effect and losartan did not. The results suggest that captopril’s effect on collateral growth in SHR is mediated by its antioxidant properties and requires an intact NO system. In addition, there is a natural suppression of local RAS components in the shear-loaded collateral vessels.

MATERIALS AND METHODS

Animals and groups. Male SHR were obtained from Harlan (Indianapolis, IN) and studied at ~10 wk of age. All procedures were approved by the School of Medicine Institutional Animal Care and Use Committee. Rats were randomly designated for study of collateral growth or for telemetric measurement of blood pressure. Subgroups received different drug therapy. Captopril and apocynin were obtained from Fisher Scientific; tempol, N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME), and enalapril were obtained from Sigma. PD-123319 and losartan were gifts from Pfizer and Merck, respectively. All drugs were given in drinking water, except for PD-123319, which was administered via osmotic minipump (Alzet; model 2ML2). Drug delivery methods and concentrations/doses were based on published studies that have demonstrated effectiveness in reducing oxidative stress (6, 51, 66, 68) or affecting arterial or ventricular remodeling (7, 14, 28, 32, 41, 46). Concentrations in drinking water (mM) were 9.2 captopril, 1.0 and 5.0 tempol, 1.5 and 3.0 apocynin, 0.2 and 0.6 losartan, and 0.6 enalapril. In some experiments, L-NAME (0.1 mM) was added along with captopril in drinking water. For delivery of PD-123319 (~30 mg\textperiodcentered kg\textsuperscript{-1}\textperiodcentered day\textsuperscript{-1}), the osmotic minipump was pre-filled with PD-123319 (62.5 mg/ml) under sterile conditions before implantation. After anesthesia and with aseptic technique, the minipump was placed in a subcutaneous pocket in the nape of the neck.

Model of collateral growth and its assessment. We utilized our model of mesenteric artery collateral growth, as previously described (59). Advantages of this model over the femoral artery ligation model include the clearly defined collateral pathway and the ability to make repeated measurements of in vivo arterial diameters. Tuttle et al. (59) have previously demonstrated with this model that collateral growth occurs in normotensive control animals, but not SHR. Surgical depth anesthesia was achieved with isoflurane, and an antibiotic was administered (cephazolin, 15 mg/kg sc). Then, with the use of aseptic technique, a laparotomy was performed, and the terminal ileum exteriorized into a heated tissue support chamber. The bowel and mesentery were immersed in phosphate-buffered saline or covered with plastic wrap at all times. As illustrated in Fig. 1A, several

![Fig. 1. Mesenteric model of collateral artery growth.](http://ajpheart.physiology.org/)

A: a region of the terminal ileum is selected so that ligation of 3–4 sequential ileal arteries will create a collateral-dependent pathway with 40–50 microvascular perfusion units (first-order arterioles) between the two collateral vessels. Two arteries are also selected as same-animal controls. Great care is taken to ligate only the arteries, leaving companion veins intact. Digital images are then made at specific locations on each control and collateral artery under dilated conditions and repeated 7 days later. Paired images from each artery as depicted in B are later used for inner diameter measurement (red cell column) and calculation of percent change in inner diameter.
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sequential ileal arteries were ligated with 9-0 monofilament suture, such that a region of bowel containing ~45 microvascular perfusion units was dependent on collateral arteries for perfusion. The suflation solution was then replaced with one containing 1.0 mM adenosine and 0.1 mM sodium nitroprusside to dilate the vasculature. Digital images of collateral and same-animal control arteries were acquired at maximum magnification with a dissecting microscope (Leica MZ 9.5) and camera (Spot Insight 4 Firewire). The bowel was returned to the abdominal cavity, and the incision was closed in two layers with 4-0 suture. Pain medication was administered for 2 days (buprinorphine 0.01 mg/kg sc bid). One week later, laparotomy and acquisition of digital images were repeated. Representative digital images are shown in Fig. 1B. The red cell column in arteries was measured as inner arterial diameter with image analysis software (Image-J), and the percent change in diameter was calculated. For assessment of collateral growth, drug therapy was initiated 3-5 days before model creation and continued until final experimentation.

Telemetry measurements of arterial pressure. Under general inhaled anesthesia, the right groin was shaved and prepped. Using aseptic technique, a 2-cm 4-0 silk incision was made, and blunt forceps were used to expose the femoral artery, vein, and nerve. The femoral artery was carefully isolated from the vein and nerve, ligated distally with 4-0 silk suture, and clamped proximally with a microvascular clamp. A small arteriotomy was created in the femoral artery. The catheter of the TA11PA-C40 transmitter (Data Sciences International, St. Paul, MN) was introduced through this arteriotomy. The catheter was advanced antegrade ~5 cm such that the tip of the catheter rested in the abdominal aorta. Another 4-0 silk suture was tied around the femoral artery, just proximal to the entrance of the catheter, to secure the catheter in place. Blunt dissection through the same incision was used to create a subcutaneous pocket in the flank for housing of the transmitter. The transmitter was placed, and the subcutaneous layer was closed to seal the pocket using a running 4-0 Polysorb suture. The skin incision then was closed. Animals were allowed to recover for 2–3 days, at which time initial 24-h recordings of heart rate, systolic, mean, and diastolic blood pressure were obtained, utilizing DSI receivers and software. A second set of recordings were obtained 3–4 days after animals were started on drug therapy. The third (final) set of recordings was obtained 7 days after initiation of drug therapy.

Quantitative RT-PCR. Relative differences in mRNA expression were determined by using real-time quantitative PCR. For vessel isolation, the abdominal aorta of an anesthetized rat was cannulated above the iliac bifurcation. After ligating both renal arteries and the proximal aorta, the mesenteric circulation was perfused with 30 ml of cold, phosphate-buffered saline followed by 10 ml of RNAlater (Ambion, Austin, TX). Isolated mesenteric arteries were excised and preserved in RNAlater at −20°C before RNA isolation. Tissues were weighed, disrupted by using a bead homogenizer (FastPrep System; QBIogene, Carlsbad, CA), and total RNA was purified by using an RNeasy Fibrous Tissue Mini Kit (Qiagen, Valencia, CA). To ensure high-quality RNA, sample concentration and integrity were determined by using 260- to 280-nm absorbance ratio and by analysis with an Agilent 2100 Bioanalyzer (RNA 6000 Nano Chip Kit). Aliquots of purified total RNA (0.5 μg) were treated to remove contaminating genomic DNA (DNA-free; Ambion) and then reverse transcribed using Ready-To-Go You Prime First Strand Beads (GE Healthcare/Amersham Biosciences, Piscataway, NJ) with random decamer priming. For PCR, an aliquot of cDNA (5.0 μl of 1:5 to 1:50 dilution) was combined with the appropriate primers for the target or β-actin endogenous control (TaqMan Gene Expression Assays; Applied Biosystems, Foster City, CA) in the presence of PCR reagents (QuantiTec Probe PCR Kit; Qiagen). Reactions were run in triplicate on an Applied Biosystems 7500 Real-Time PCR System using relative quantification (ddCt, where Ct is cycle threshold) with dual-labeled (FAM/MGB) probes as the product detection method. Standard 7500 PCR cycling conditions were used for angiotensinogen (Agt), but modified to 45 cycles for AT1R experiments. Differences in PCR product yields between groups were determined by comparing the fold differences between target mRNA after normalization to β-actin.

Statistical analyses. Tests for statistical significance were performed with SigmaStat 3.0. Repeated-measures ANOVA was used with multiple pairwise comparisons performed with the Holm-Sidak method. Data are presented as group averages with SE of the mean. The correlation between changes in collateral artery diameters and arterial pressure was evaluated with linear regression analysis.

RESULTS

Captopril reverses the impairment of collateral growth. The maximally dilated inner diameters of control and collateral arteries were similar within groups, averaging 267 ± 5.1 μm at initial model creation. Measurements of the same vessel diameters from time of ligation to 1 wk later were made for control and collateral arteries in all groups. Changes in maximally dilated inner diameters for the various treatment groups are reported as percent diameter change in Fig. 2. No significant increases occurred in the control arteries of any group. The diameters of the collateral arteries in untreated (control) SHR were not increased, confirming our laboratory’s earlier study of collateral growth in SHR (59). In captopril-treated SHR, a remarkable increase (141 ± 13.6 μm; P < 0.001) occurred in collaterals.

Tempol and apocynin, but not losartan, enhance collateral growth. To investigate the mechanisms by which captopril reversed SHR collateral growth impairment, the effect of commonly used chronic doses of tempol, apocynin, and losartan were evaluated for their effect on collateral growth. The results are summarized in Fig. 2B. Statistically significant enlargement of the collateral arteries did not occur for any of these groups at the initial dose tested. There was, however, a tendency for collateral diameter enlargement with both tempol and apocynin (29 ± 13 and 39 ± 15 μm increase, respectively). Subsequent experiments were performed with increased concentrations (5 mM for tempol, 3 mM for apocynin). These higher concentrations resulted in statistically significant increases in collateral diameters, 88 ± 14 (P = 0.006) and 77 ± 14 μm (P < 0.001), respectively, for tempol and apocynin. These diameter increases were not statistically different from those observed with captopril. A higher dose of losartan was also tested, but did not promote collateral growth.

Enalapril and PD-123319 do not promote collateral growth; l-NAME inhibits captopril’s enhancement of collateral growth. After the unexpected results with losartan, additional experiments were performed to clarify the mechanisms by which captopril, but not losartan, promoted collateral growth. ACEI can influence remodeling events by their effect on the kalikrein-kinin system and stimulation of NO production (15). The sulfhydryl group within captopril endows it with antioxidant properties (38) not found in most ACEI. The AT2R may also impact vascular structure and remodeling (32). To address these possibilities, we investigated the effect on collateral growth of enalapril as an alternative ACEI without the antioxidant potential of captopril (38, 52), the selective AT2R antagonist PD-123319, and low-dose l-NAME in combination with captopril. The results are presented in Fig. 2C. None of these treatments resulted in significant collateral growth. The lack of an enalapril effect suggests that captopril’s enhancement of collateral growth is not solely mediated by inhibition of angiotensin-converting enzyme. The absence of an effect of
inward remodeling, which could reduce the capacity for flow-induced outward remodeling. A secondary objective was to verify that the doses of enalapril and losartan, which had no effect on collateral growth, were indeed effective in reducing arterial pressure. Average 24-h arterial pressure in young untreated SHR was 135 ± 1.9 mmHg. Captopril and enalapril produced equivalent reductions in arterial pressure (30 ± 1.1 vs. 28 ± 2.6%). Both doses of losartan produced similar pressure reductions, averaging 16 ± 2.5%. Mean arterial pressure was also significantly reduced by the highest dose of tempol. The pressure reductions obtained with losartan and tempol were less than captopril. Treatment with the higher dose of apocynin did not significantly decrease arterial pressure (4 ± 0.7%). The potential correlation between pressure reduction and collateral growth was evaluated with regression analysis of changes in arterial pressure and collateral growth (Fig. 3).

**Fig. 3.** Correlation of the increase in collateral diameter with decrease in mean arterial pressure (MAP). Regression analysis was performed from averages obtained for collateral growth (CG) and blood pressure measurement by telemetry in different groups of animals and for various drugs. The slope of the regression line was not significantly different than zero (P = 0.222), and the correlation coefficient was very low (0.007). The results with enalapril demonstrate that pressure reduction alone is not sufficient to stimulate collateral growth. In addition, the data for apocynin establish that collateral growth can occur in the presence of hypertension.

**Fig. 2.** Effect of drug treatment on collateral growth. A–C: the percent change in maximally dilated in vivo inner diameter of control and collateral arteries for the various treatments is shown. During the week between measurements, there were no significant changes in diameter of control arteries. The diameter of collaterals was increased only after treatment with captopril, tempol, and apocynin. N = 4–6.

PD-123319 at a dose previously shown to prevent ANG II-induced AT2R-dependent vascular remodeling (32) indicates that the beneficial effect of captopril is not mediated through the AT2R. The prevention of captopril’s stimulation of collateral growth by low-dose l-NAME indicates that captopril’s action is mediated by or at least requires NO, consistent with the mechanisms by which captopril has been shown to inhibit intimal hyperplasia (15).

**Collateral growth enhancement is independent of arterial pressure reduction.** Arterial pressures were measured by telemetry in separate animals receiving identical drug treatments. This was done primarily to determine the effect of blood pressure reduction on collateral growth. Hypertension itself can induce superoxide production, medial hypertrophy, and inward remodeling, which could reduce the capacity for flow-induced outward remodeling. A secondary objective was to verify that the doses of enalapril and losartan, which had no effect on collateral growth, were indeed effective in reducing arterial pressure. Average 24-h arterial pressure in young untreated SHR was 135 ± 1.9 mmHg. Captopril and enalapril produced equivalent reductions in arterial pressure (30 ± 1.1 vs. 28 ± 2.6%). Both doses of losartan produced similar pressure reductions, averaging 16 ± 2.5%. Mean arterial pressure was also significantly reduced by the highest dose of tempol. The pressure reductions obtained with losartan and tempol were less than captopril. Treatment with the higher dose of apocynin did not significantly decrease arterial pressure (4 ± 0.7%). The potential correlation between pressure reduction and collateral growth was evaluated with regression analysis of changes in arterial pressure and collateral growth (Fig. 3).

**The local RAS system is suppressed in SHR collaterals.** With the understanding that ANG II and AT1R levels and NAD(P)H oxidase expression are increased in the young SHR (6, 40, 47, 50) and that ANG II activates NAD(P)H oxidase via the AT1R (29), we were without an explanation for why blockade of the AT1R by losartan did not promote collateral growth. Inspection of preliminary genomic data from our laboratory obtained with microarray analysis revealed a trend for some components of the RAS to be downregulated in SHR collaterals. Since this could potentially explain the lack of an effect of losartan as well as enalapril on collateral growth, RT-PCR experiments were performed 1 and 3 days after arterial ligation to evaluate AT1R and Agt mRNA expression. By day 3 in our model, cell proliferation had begun and luminal expansion was occurring (57). Collateral arteries were compared with the same animal control vessels. The data indicate that expression levels of both molecules were consistently reduced in collaterals (Fig. 4).

**Fig. 2.** Effect of drug treatment on collateral growth. A–C: the percent change in maximally dilated in vivo inner diameter of control and collateral arteries for the various treatments is shown. During the week between measurements, there were no significant changes in diameter of control arteries. The diameter of collaterals was increased only after treatment with captopril, tempol, and apocynin. N = 4–6.
enhancement involves collateralization of existing vessels rather than angiogenesis or neovascularization. A similar observation with ACEI has been reported for patients with coronary artery disease (35). Based on the known phenotype of SHR and the effects of captopril, we hypothesized that SHR collateral growth impairment is mediated by excess superoxide produced by NAD(P)H oxidase in response to stimulation of the AT1R by ANG II. Because pressure elevation can increase oxidant production (39) and initiate medial hypertrophy and inward remodeling in resistance vessels (73), we were also interested in determining whether pressure reduction was important for the outward remodeling associated with collateral growth.

The results are consistent with our hypothesis regarding a role for excess superoxide produced by NAD(P)H oxidase in SHR collateral growth impairment. Chronic administration of the SOD mimetic tempol restored the capacity for collateral growth in a dose-dependent manner (Fig. 2B). Other studies have shown chronic administration of tempol to SHR reduces oxidative stress, arterial pressure, and microvascular rarefaction and increases NO bioavailability (28, 51, 66, 68). Apocynin is a widely used inhibitor of NAD(P)H oxidase. In vivo studies with chronic apocynin administration (1.5–2.5 mM in drinking water) have shown decreased oxidative stress in SHR (41) and attenuation of aldosterone-induced elevation of NAD(P)H oxidase, arterial pressure, p22phox expression, and cardiac remodeling (42). In our study, apocynin also promoted collateral growth in a dose-dependent manner (Fig. 2B), implicating a significant role of NAD(P)H oxidase in the impairment of SHR collateral growth. These results suggest that antioxidant therapy might be effective as either primary or adjuvant therapy to improve vascular compensation to arterial insufficiency, specifically collateral growth. While completed clinical trials with antioxidants have revealed no significant benefit related to cardiovascular health, significant flaws of these trials have been identified (21, 55). These shortcomings include the lack of prescreening to identify patients with elevated oxidative stress and the utilization of ineffective antioxidant therapies, such as vitamins C and E.

Unlike the results with tempol and apocynin, the effects of losartan and enalapril (Fig. 2) were the opposite of what we anticipated. Neither was effective in promoting collateral growth. In contrast, the previously cited study in the SHR hindlimb (17) demonstrated improved perfusion after femoral artery ligation in response to ramipril, a nonsulphydryl ACEI. In addition, the clinical study that demonstrated significant benefit of ACEI related to collateral growth in patients with coronary artery disease excluded patients receiving captopril (35). There are several potential explanations for these disparities. In the ramipril study (17), therapy was administered immediately after rather than before arterial ligation and was continued for 28 days vs. 7 days in the present study. It is possible that longer term therapy with losartan and enalapril would have been effective in our study. However, other investigations have revealed differences between various ACEI and ARBs for specific outcomes (4, 20). Zofenopril, another sulfhydryl ACEI, but not enalapril, reduced oxidative stress in patients with essential hypertension (38). However, other studies, including one in SHR (33), have shown enalapril to be effective in reducing oxidative stress. In a rabbit hindlimb ischemia model, quinaprilat was much more effective in pro-

**Fig. 4.** mRNA expression of angiotensin II type 1 receptor (AT1R) and angiotensigen (Agt). Relative mRNA expression of AT1R and Agt was determined by real-time RT-PCR for whole vessel lysates of control and collateral arteries and normalized to ß-actin. The relative expression in the collateral was then normalized to the same-animal control, and the means and SEs are reported in the figure. The 1- and 3-day time points were evaluated to determine whether expression was altered before and after the collateral had begun to expand (57). N = 4 for 1- and 3-day time points.

**DISCUSSION**

This study was performed in an animal model of vascular disease characterized by impaired collateral artery growth. To our knowledge, these results are the first to demonstrate the capability of antioxidant therapy to reverse impaired collateral growth in a model of endothelial dysfunction. Because all of the conditions that are associated with impaired collateral growth (hypertension, diabetes, hypercholesterolemia, and aging) in patients (1, 5, 23, 27, 37) and animals (11, 44, 45, 49, 58, 59, 63) are associated with elevated oxidative stress, we believe that this is an important finding. In addition, a novel observation is the suppression of local RAS components in developing collateral vessels.

In hypertension, oxidative stress may be increased by multiple enzyme systems, including xanthine oxidase, uncoupled eNOS, the mitochondrial respiratory chain, and NAD(P)H oxidase (30). In male SHR, superoxide production by vascular NAD(P)H oxidase is increased (22, 60). NAD(P)H oxidase expression/activation is regulated by many factors, including endothelin and angiotensin. ANG II vascular levels may be 10× greater than in plasma in normotensive rats and even higher in SHR (3). Activation of the AT1R by ANG II results in activation of NAD(P)H oxidase and elevation of superoxide formation (29). Captopril, at a comparable dose used in this study, reduces SHR arterial pressures, plasma angiotensin and endothelin, and oxidative stress (6). Our initial results confirmed earlier studies of impaired adaptation to arterial occlusion in SHR (16, 17, 59) and demonstrated a remarkable effect of captopril on collateral growth (51 ± 8.7% luminal expansion in 7 days, Fig. 2A). The collateral growth observed with captopril treatment was equivalent to or greater than what we have previously observed in young normotensive rats (59, 61). These results, together with the earlier report demonstrating that ramipril improves perfusion in the hindlimb ligation model (17), clearly establish the therapeutic capacity of ACEI to promote vascular compensation in the SHR. The lack of an increase in capillary or arteriolar density in the earlier report and the clear demonstration of luminal expansion of preexisting bypass vessels in the present study suggest that the ACEI...
moting angiogenesis and inhibiting tissue ACE than captopril (18). In experimental tumor growth, captopril, but not lisinopril or enalaprilat, inhibits angiogenesis (64). Such findings may be related to differences in ACEI properties [e.g., potency, bioavailability, distribution and affinity for tissue ACE (8), altered receptor distributions (31), and/or changes in levels of ANG II and ANG(1-7) (19, 25)]. These diverse outcomes for different RAS suppressing drugs illustrate the need for additional study.

Another unexpected observation was the suppression of the local RAS in mesenteric collaterals (Fig. 4). This novel finding of reduced mRNA levels of AT1R and Agt is consistent with previous studies, which have shown the existence of a vascular RAS and the downregulation of RAS components by shear stress and/or NO. Functional evidence of a local microvascular RAS was reported in 1994 by Vicaut and Hou (63a). In an isolated cremaster preparation, greater arteriolar vasoconstriction to Agt and ANG I was observed in SHR than in Wistar Kyoto rats, and this vasoconstriction was prevented by ACEI. More recently, Agoudemos and Greene (3) have demonstrated the presence of Agt and renin mRNA and protein in rat skeletal muscle arterioles. Tissue ANG II levels were more than 10× greater than plasma levels in normotensive animals and even higher in SHR (3). Greene’s group has also shown that shear stress elevation alters endothelial cell function by suppressing ACE gene and protein expression in vitro and in vivo (43). More recent studies by other groups indicate that both ACE and additional RAS components are downregulated by shear stress or NO. Miyakawa et al. (36) found two novel shear stress response elements in the rat ACE promoter, which downregulate ACE expression when shear is elevated. A flow or shear stress-dependent suppression of AT1R is also consistent with the finding of Adams et al. (2) that physical exercise is associated with downregulation of this receptor in human internal mammary arteries. Yan et al. (67) have reviewed mechanisms by which NO may regulate the ANG II signaling pathway, including reduction of ACE activity and AT1R expression. Thus evidence exists in other systems and experimental conditions to support the hypothesis that elevated shear

Fig. 5. Proposed interactions between the renin angiotensin system, NADPH oxidase, oxidative stress, and nitric oxide (NO) bioavailability that influence collateral growth. Our central hypothesis has been that the balance between NO and superoxide levels determines in large part whether collateral growth is successful or impaired. Local levels of NO and O$_2^-$ can impact collateral growth by altering the expression and activity of growth modulators and matrix metalloproteases and function of bone marrow-derived cells. In the spontaneously hypertensive rat and other models of endothelial dysfunction characterized by elevated angiotensin II and/or NAD(P)H oxidase, elevated levels of superoxide or daughter compounds can impair growth through multiple mechanisms. In this schematic, solid and dashed arrows represent stimulation and inhibition, respectively. The schematic emphasizes how elevated shear and/or NO production in collateral vessels may suppress components of the local renin angiotensin system. eNOS, endothelial NO synthase; ACE, angiotensin-converting enzyme; AT1 and AT2, angiotensin II receptor types 1 and 2, respectively.
stress and/or NO suppresses the local vascular RAS. However, additional studies are needed to confirm the functional relevance of the mRNA suppression and establish its role on collateral growth in this and other models.

The results suggest that suppression of the local RAS, together with reduction of oxidative stress in SHR, are essential for the successful outward remodeling of collateral vessels. The inward remodeling of mesenteric resistance arteries in young SHR is prevented or reversed by chronic suppression of the RAS with enalapril and losartan (46). Our observations of reduced Agt and AT1R expression in SHR collaterals support this hypothesis and also provide a potential explanation for the systemic effect of enalapril and losartan on arterial pressure without a beneficial effect on collateral growth.

Although ACEI and ARB are widely prescribed for patients with vascular disease, the specific role of the vascular RAS in tumor angiogenesis (13, 64) and adaptive responses to arterial insufficiency (12, 17, 18, 35, 48, 54) remain controversial. It is clear from existing studies that there are multiple mechanisms/pathways through which RAS may promote and suppress vascular remodeling. Even within different vascular beds of the same animal, RAS-suppressing drugs can have opposing effects (12). Recent studies have shown that ANG(1-7) may oppose actions of ANG II. Also, the hematopoietic stem cell regulator N-acetyl-seryl-aspartyl-lysyl-proline promotes angiogenesis (65) and is modulated by ACEI, including captopril (26). These recent observations, together with contradictory studies, emphasize the need for additional studies to clarify mechanisms by which angiotensin and RAS-suppressing drugs influence vascular remodeling, including that which occurs in response to arterial occlusion.

While we believe this study has potentially important clinical significance, there are a number of limitations with our study that should be considered. First, therapy was initiated before arterial insufficiency was created. This approach was taken, because our objective was to determine the mechanisms responsible for collateral growth impairment in young SHR rather than ways to promote collateral growth. While the previous work by Emanueli et al. (17) demonstrates that therapy can be effective when given immediately after abrupt ligation, future work is needed to determine whether similar therapies can reverse a longstanding impaired response. As already indicated, our treatment duration was short term. Other therapies may be just as effective when administered for a longer duration. Also, we studied young rats during the developmental stage of hypertension. We anticipate that therapy would be effective in established hypertension, but additional pathologic development with aging and long-term hypertension, which may reduce or limit effectiveness. It is also possible that responses in mesenteric collaterals do not reflect what occurs in other organs. However, our results are consistent with studies that have shown ACEI to increase hindlimb perfusion after arterial ligation (17, 70) without stimulating angiogenesis (17).

In conclusion, we have demonstrated that impaired collateral growth in SHR can be dramatically reversed with antioxidant therapy. The results are consistent with the hypothesis that elevated NO levels are required for successful collateral growth and that excess superoxide produced from NAD(P)H oxidase impairs collateral growth by suppressing bioavailable NO. Downregulation of the local RAS may also be important for collateral growth. A schematic is presented in Fig. 5 to illustrate how these components may interact to influence collateral growth. The role of the RAS in angiogenesis and arteriogenesis is controversial, and the results from this study raise questions about the importance of suppressing the RAS to promote collateral growth. Given the widespread use of ACEI and ARB, this remains an important area for investigation.

ACKNOWLEDGMENTS

The excellent technical assistance from Randal G. Bills and P. Melanie Pride is gratefully acknowledged, as is the proof editing by Tonia Miller.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-42898.

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Oxidant Stress and SHR Collateral Growth Impairment


artery remodeling: wall dimensions, cell density, and eNOS expression.


