Basic fibroblast growth factor increases collateral blood flow in spontaneously hypertensive rats

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1Section of Vascular Surgery, University of Michigan, Ann Arbor, Michigan 48109; and 2Department of Biomedical Sciences, College of Veterinary Medicine, 3Department of Medical Pharmacology and Physiology, College of Medicine, and 4Dalton Cardiovascular Research Center, University of Missouri, Columbia, Missouri 65211

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Srivastava, Sunita, Ronald L. Terjung, and H. T. Yang. Basic fibroblast growth factor increases collateral blood flow in spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 285: H1190–H1197, 2003. First published May 22, 2003; 10.1152/ajpheart.00280.2003.—Ischemia-induced angiogenic response is reduced in spontaneously hypertensive rats (SHR). To study whether exogenous basic fibroblast growth factor (bFGF) infusion is effective in expanding collateral circulation in frankly hypertensive SHR, femoral arteries of male SHR (weighing ~250 g) were kept intact (nonoccluded control; n = 9) or occluded for 4 h (n = 12) or for 16 days with vehicle (n = 14) or bFGF [0.5 (n = 17), 5.0 (n = 13), and 50.0 (n = 14) μg·kg−1·day−1 for 14 days] intraarterially. Maximal collateral-dependent blood flows (BF) to the hindlimbs were determined with85Sr- and 141Ce-labeled microspheres during running at 20 and 25 m/min (15% grade). Preexercise heart rates (~530 beats/min) and blood pressures (BP; ~200 mmHg) were similar across groups except in the high-dose bFGF group, where BP was reduced by ~12% (P < 0.05). Femoral artery occlusion for 4 h resulted in ~95% reduction of BF in calf muscles [199 ± 18.7 (nonoccluded group) to 10 ± 1.0 ml·min−1·100 g−1, P < 0.001). BF to calf muscles of the vehicle and low-dose bFGF (0.5 μg·kg−1·day−1) groups increased to 36 ± 3.2 and 45 ± 2.0 ml·min−1·100 g−1, respectively (P < 0.001). bFGF infusion at 5.0 and 50.0 μg·kg−1·day−1 further increased (P < 0.001) BF to calf muscles (62 ± 4.6 and 62 ± 2.2 ml·min−1·100 g−1, respectively). Our results show that bFGF can effectively increase BF in hypertensive rats. The reduced hypertension with high-dose bFGF suggests that a critical signal in arteriogenesis (nitric oxide bioavailability) may be restored. These findings suggest that the dullest endothelial nitric oxide synthase of SHR does not prevent collateral vessel remodeling.

angiogenesis; vascular remodeling; peripheral arterial insufficiency; nitric oxide; arterial occlusion

PERIPHERAL ARTERIAL OCCLUSIVE disease (PAD) is a debilitating cardiovascular disorder that greatly affects the health and quality of life of patients. In addition to age, smoking, diabetes mellitus, and atherosclerosis, hypertension has been identified as one of the major risk factors for PAD. The most convincing epidemiological evidence linking hypertension to PAD was derived from the Framingham study (25). Kannel and McGee (25) reported that hypertension carried a 2.5-fold age-adjusted risk in men and a 3.9-fold age-adjusted risk in women to develop PAD. Moreover, a large portion of PAD patients are hypertensive. In a defined elderly population, 83–88% of the male and female PAD patients had hypertension (33) and exhibited higher incidences of coronary heart disease or cerebral vascular disease (4). Hypertensive patients with PAD had significantly higher myocardial hypertrophy, pulse pressure, and natriuresis compared with similar hypertensive patients without PAD (9). The above-mentioned factors might contribute to the higher mortality rate in hypertensive patients with PAD (15).

Enhancement of collateral circulation to ischemic tissues by means of therapeutic angiogenesis has been advocated in recent years. Angiogenic growth factors are strong candidates as potential therapeutic agents to treat PAD patients. Basic fibroblast growth factor (bFGF; FGF-2) is a potent angiogenic cytokine that induces proliferation of smooth muscle and endothelial cells, engraves collateral vessels (5, 7, 8, 37, 46), and improves collateral blood flow to active muscle (42–44, 46) after experimental peripheral artery occlusion. This improved collateral blood flow enhances muscle performance (41, 42, 46) and reduces tissue necrosis (7, 37), and it results from vascular remodeling of preexisting conduit vessels, a process termed arteriogenesis by Schaper and coworkers (10, 24). Although the exact mechanisms of inducing vascular remodeling by angiogenic growth factors are not fully understood, normal endothelial nitric oxide (NO) production is essential for bFGF-stimulated collateral vascular expansion (49).

Extensive research evidence indicates that the angiogenic response is reduced in hypertensive animals and humans. Loss of arterioles and capillaries by raffection has been found in nearly all animal models of hypertension (11, 23). Interestingly, the restoration of an impaired angiogenic response to ischemia, found in spontaneously hypertensive rats (SHR), by angiotensin-converting enzyme (ACE) inhibition (38) and kalikrein gene transfer (16) implicates the importance of NO in this process. ACE inhibition reduces degra-
tion of Bradykinin (cf. Ref. 17), and upregulation of kallikrein increases tissue kinin/bradykinin accumulation (16). Bradykinin is a potent activator of the l-arginine-NO pathway after activation of B2 kinin receptors (31, 50). Kinin/bradykinin is thought to increase the bioavailability of NO, improve the mitogenic function of the endothelium, and restore the impaired angiogenic response to ischemia (16, 17). Furthermore, the importance of NO in angiogenesis was recognized in the ischemic hindlimbs of endothelial NO synthase (eNOS)-knockout mice (32), which exhibit a reduced angiogenic capacity. This evidence indicates that NO has a critical role in vascular remodeling. At present, it is unknown whether exogenous bFGF can effect collateral expansion in the presence of hypertension, likely related to an impaired NOS-NO system, as is thought to be the case for SHR. Cuevas et al. (14) reported that FGF content and eNOS activity were reduced in SHR during development. Restoring FGF content in the arterial endothelium increased eNOS content and lowered hypertension (14). Therefore, in the present study, we tested the efficacy of exogenous bFGF to enhance collateral blood flow in SHR with experimental bilateral occlusion of the femoral arteries.

METHODS

Experiment design. The experimental design permitted evaluation of bFGF effects on collateral-dependent blood flow to the hindlimb. Frankly hypertensive SHR had normal hindlimb circulation (nonoccluded control group; n = 9) or received bilateral occlusion of femoral arteries either for 4 h (acutely occluded group; n = 12) or 16 days (chronically occluded animals; n = 58). The chronically occluded animals were further divided into the following treatment groups: vehicle (n = 14) or bFGF at doses of 0.5 (low dose; n = 17), 5.0 (mid dose; n = 13), and 50.0 (high dose; n = 14) μg·kg⁻¹·day⁻¹. bFGF or vehicle infusion started at the time of femoral artery occlusion via a 14-day osmotic pump. At ~12 wk of age, collateral-dependent blood flow was determined during treadmill running. This was at day 16 after femoral artery occlusion and 2 days after the end of bFGF infusion, an extensive time for clearance of bFGF from the circulation.

Animal care. Male ~10-wk-old SHR weighing ~225 g (Taconic Farms, Germantown, NY) were housed two per cage in a temperature (21°C) and light 12:12-h light-dark cycle-controlled room. Rats were fed Purina Rat Chow and water ad libitum. On arrival, all rats were acclimated to handling and treadmill (Quinton model 42-15) walking at 20 m/min on a 15% grade for 5–10 min daily for ~5 days. The treadmill protocol included briefly turning the treadmill on and off to condition the rats to run at the front of the treadmill when the belt started moving. This treadmill conditioning protocol does not produce any detectable peripheral adaptations in the animals, as shown in our previous experiments (48).

The care and treatment of animals and all experimental procedures were carried out in accordance with NIH guidelines and were approved by the Animal Care and Use Committee of the University of Missouri, Columbia, MO.

Surgical preparation for femoral artery occlusion and growth factor infusion. The details of the procedure were described in our previous reports (43, 44, 48). In brief, under ketamine-acepromazine (100 mg·0.5 mg⁻¹·kg body wt⁻¹) anesthesia, both femoral arteries were surgically exposed and occluded with 3-0 surgical silk sutures ~5 mm distal to the inguinal ligament. In addition, a PE-60 catheter connected to an osmotic pump (Alzet model 2002; Alza, Palo Alto, CA) was inserted into the left common iliac artery through the occluded femoral artery for infusion of bFGF (Scios, Sunnyvale, CA) into the flow delivered to the internal iliac artery. Each osmotic pump was subsequently checked to verify complete delivery of its volume. Topical antibiotic powder (Neo-Predef; Upjohn, Kalamazoo, MI) was placed on the wound before closure with skin clips.

Osmotic pump preparation was conducted according to the instructions of Alza. The miniosmotic pumps were designed for constant delivery at a flow rate of 0.50 ± 0.02 μl/h for 14 days. The pumps were filled with either vehicle solution (10% sodium citrate and 1.6% glycerol in phosphate-buffered saline) or vehicle plus bFGF at one of three doses (0.5, 5.0, and 50.0 μg·kg⁻¹·day⁻¹). The dead space of the femoral catheter was filled with the same solution as in its pump. The pump was housed in a tunnel under the skin in the left groin area; this placement did not hamper hindlimb movement while rats were walking on the treadmill.

Blood flow determination. On day 16 after pump installation and femoral artery occlusion, rats from each group were surgically prepared for blood flow measurement under ketamine anesthesia as done previously (43, 44, 48). Briefly, a PE-50 catheter was placed in the left carotid artery and advanced to the arch of the aorta for monitoring blood pressure and heart rate as well as for infusing microspheres. A second catheter was placed in the caudal artery for monitoring caudal blood pressure and obtaining the reference blood sample during microsphere infusion. The arteries were catheterized early in the morning. Blood flow determinations during treadmill running were made in fully recovered animals after ~4 h recovery, as described previously (48).

Muscle blood flows were determined by using radiolabeled microspheres (⁸⁵Sr and ¹⁴¹Ce, 15 ± 0.1 μm diameter; NEN, Boston, MA) during the second minute of running at both a low (20 m/min, 15% grade) and a high (25 m/min, 15% grade) speed. The higher running speed ensured that maximal collateral-dependent blood flow was achieved. Blood flow to nonischemic active muscle is proportional to running intensity (3); however, maximal blood flow to collateral-dependent muscle is controlled by upstream resistance of the collateral circuit when the downstream resistance in the active muscle is minimal (45, 47, 48). Minimal muscular resistance was achieved when blood flow to the collateral-dependent muscle did not increase further at the higher running intensity. This observation was found in our previous studies (43–45, 47, 48). At the end of the first minute of running at each speed, a well-mixed suspension of microspheres was carefully infused through the catheter in the carotid artery, followed by a 0.5-ml saline flush over ~20 s. At the same time, a reference blood sample was withdrawn from the caudal artery at a rate of 0.5 ml/min, beginning 10 s before each microsphere infusion and continuing for 90 s. After the second microsphere infusion, animals were killed by an overdose of pentobarbital sodium. Tissue samples dissected from both hindlimbs, together with the reference blood flow sample, were counted with an autogamma counter (Wallac Wizard 1480 Autogamma-counter; Turku, Finland). Muscle blood flow (ml·min⁻¹·100 g⁻¹) was calculated as:

\[
\text{blood flow} = (0.5 \text{ ml/min} \times \text{CPM}_{\text{RBS}}^{-1}) \times (\text{CPM}_{\text{tissue}} \times \text{tissue wt}^{-1}) \times 100
\]

where RBS is the reference blood sample and CPM is counts per minute. Results from both hindlimbs were averaged after
it was determined that there were no significant differences in blood flows between corresponding left and right hindlimb tissues. Furthermore, comparison of kidney blood flows within each animal provided evidence of proper mixing of microspheres. Blood flows to individual tissue sections of the hindlimb were summed to assess blood flows to the total, proximal, and distal regions, as done previously (48).

Data analysis. All data are expressed as means ± SE. Analyses of variance were used to assess the main treatment effects of bFGF. P < 0.05 was recognized as a significant difference. The treatment differences across groups were determined by Tukey’s procedure (36).

RESULTS

General responses to treatment. All animals tolerated the procedure of bilateral occlusion of the femoral arteries well and with a 100% success rate. There were no signs of limb ischemia or necrosis and no presence of infection or complications from the osmotic pumps in the occluded animals. The residual solution within the osmotic pumps was minimal (10–20 μl left), confirming the effectiveness of pump delivery and the patency of the pump catheter.

The body weight of nonoccluded control animals was lower than those animals from the 0.5 and 50.0 μg bFGF groups (P < 0.05). In addition, the weights of total, proximal, and distal hindlimbs of the nonoccluded control group were higher than those of the vehicle and 5.0 μg bFGF groups (P < 0.05; Table 1). The weight of the gastrocnemius-plantaris-soleus (GPS) muscle group in the nonoccluded group was significantly different from the nonoccluded control group by Tukey’s post hoc test (P < 0.05).

Table 1. Body and hindlimb tissue weight in male spontaneously hypertensive rats

<table>
<thead>
<tr>
<th></th>
<th>Nonoccluded Control (n = 9)</th>
<th>Acutely Occluded (n = 12)</th>
<th>Occluded 16 days + bFGF, μg·kg⁻¹·day⁻¹</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle (n = 14)</td>
<td>Low (n = 17)</td>
<td>Mid (n = 13)</td>
<td>High (n = 14)</td>
</tr>
<tr>
<td>Body weight</td>
<td>243 ± 11.3</td>
<td>267 ± 4.2</td>
<td>273 ± 4.9</td>
<td>290 ± 8.1*</td>
</tr>
<tr>
<td>Hindlimb weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15.4 ± 0.30</td>
<td>13.9 ± 0.44</td>
<td>13.0 ± 0.57*</td>
<td>13.9 ± 0.40</td>
</tr>
<tr>
<td>Proximal</td>
<td>9.57 ± 0.17</td>
<td>8.36 ± 0.36</td>
<td>7.80 ± 0.41*</td>
<td>8.37 ± 0.32</td>
</tr>
<tr>
<td>Distal</td>
<td>5.71 ± 0.16</td>
<td>5.40 ± 0.12</td>
<td>5.09 ± 0.17*</td>
<td>5.39 ± 0.11</td>
</tr>
<tr>
<td>GPS group</td>
<td>1.59 ± 0.04</td>
<td>1.49 ± 0.07</td>
<td>1.35 ± 0.06</td>
<td>1.43 ± 0.04</td>
</tr>
</tbody>
</table>

Data (in grams) are expressed as means ± SE. bFGF, basic fibroblast growth factor; GPS group, gastrocnemius-plantaris-soleus muscles; occluded, occlusion of bilateral femoral artery; acutely occluded, bilateral femoral artery occlusion for 4 h. *Significantly different from nonoccluded control group by Tukey’s post hoc test (P < 0.05).

In the high-dose bFGF group, blood pressure was lower than other groups before exercise (P < 0.05) and exhibited a tendency to be lower during exercise. However, blood pressures were not different across other groups during exercise (Table 2). Treadmill running significantly lowered blood pressure (~8%; P < 0.001) in all groups except the high-dose bFGF group (Table 2). Moreover, there was no speed effect in the high-dose bFGF group (P > 0.05), because blood pressure was already low before exercise.

Heart rate was not different across the treatment groups, either before exercise or during exercise (Table 2). Treadmill exercise at both low and high speeds significantly increased heart rate compared with pre-exercise condition (P < 0.001).

Blood flow determination. There was an ~10% failure rate in completion of blood flow measurement, mainly because of difficulty in catheter placement or patency. Evenness of microsphere distribution within the animals was confirmed by similar blood flows in the left and right kidneys (1.02 ± 0.02, blood flows to the left kidney divided by those to the right kidney; total of 143 observations) and left and right hindlimbs (0.91 ± 0.02; 144 observations). Therefore, blood flow values from left and right side tissues were combined into one value for each tissue of each animal. Furthermore,

Table 2. Blood pressure and heart rate

<table>
<thead>
<tr>
<th></th>
<th>Nonoccluded Control (n = 4)</th>
<th>Acutely Occluded (n = 12)</th>
<th>Occluded 16 days + bFGF, μg·kg⁻¹·day⁻¹</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle (n = 14)</td>
<td>Low (n = 17)</td>
<td>Mid (n = 13)</td>
<td>High (n = 14)</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preexercise</td>
<td>186 ± 7.6</td>
<td>194 ± 5.5</td>
<td>205 ± 4.7</td>
<td>196 ± 5.4</td>
</tr>
<tr>
<td>20 m/min</td>
<td>169 ± 10.3†</td>
<td>184 ± 7.2</td>
<td>203 ± 6.8</td>
<td>185 ± 6.6†</td>
</tr>
<tr>
<td>25 m/min</td>
<td>170 ± 10.2†</td>
<td>175 ± 7.2†</td>
<td>190 ± 9.1†</td>
<td>188 ± 7.2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preexercise</td>
<td>538 ± 17.5</td>
<td>530 ± 10.0</td>
<td>529 ± 7.4</td>
<td>529 ± 7.7</td>
</tr>
<tr>
<td>20 m/min</td>
<td>580 ± 17.3†</td>
<td>556 ± 16.4†</td>
<td>553 ± 11.2†</td>
<td>544 ± 9.6</td>
</tr>
<tr>
<td>25 m/min</td>
<td>607 ± 12.0††</td>
<td>589 ± 7.3††</td>
<td>564 ± 9.0††</td>
<td>574 ± 7.7††</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. *Significantly different from 20 m/min value by Tukey’s post hoc test (P < 0.05); †significantly different from preexercise value; §significantly different from other occluded groups (P < 0.05). NS, not significant.
blood flows obtained at the higher running speed were not different from those at the low speed, indicating that the upstream resistance of the collateral vessels was the primary determinant of blood flow to the collateral-dependent calf muscles. Replicate blood flow determinations were not achieved in a few animals (e.g., because of unacceptable running performance at the higher running speed or catheter patency), accounting for the difference in group size ($n$) between the low- and high-speed measurements given in Table 5. Although replicate blood flows could not be verified in these animals, we have accepted the flow determination at the single speed as meaningful, because speed did not change collateral-dependent blood flow. Inclusion of these values has not altered the findings of the study.

**bFGF-induced collateral blood flow.** Femoral artery occlusion for 4 h significantly reduced exercise-induced blood flow to total hindlimb (~55%), distal hindlimb (~75%), and GPS muscle groups (~55%) compared with nonoccluded animals ($P < 0.001$; Table 3). Blood flows to the distal hindlimb and GPS muscle moderately recovered in the vehicle group after 16 days of occlusion ($P = 0.05$). bFGF infusion for 2 wk significantly increased blood flow to the total hindlimb in all three bFGF groups ($P < 0.001$) compared with the vehicle control group (Table 3). bFGF infusion at mid and high doses induced a ~60% increase in blood flow of distal hindlimb compared with the vehicle group ($P < 0.001$; Table 3). Moreover, collateral-dependent blood flow to GPS muscles (calf muscle) increased ~70% in both mid- and high-dose bFGF groups compared with the vehicle group ($P < 0.001$; Fig. 1). Accordingly, the individual muscles that comprise the distal hindlimb and GPS muscle groups showed higher blood flows with the mid- and high-dose bFGF treatment ($P < 0.001$; Table 4). The collateral-dependent blood flow to the GPS muscle group was similar between mid- and high-dose bFGF groups (Table 3, Fig. 1).

**Blood flows to kidneys and non-flow-restricted muscle tissues.** Renal blood flows were lower in all occluded groups compared with the nonoccluded control group ($P < 0.005$; Table 5). High-speed running lowered kidney blood flows across groups ($P < 0.001$). Femoral artery occlusion for 16 days (all groups) reduced blood flow in psoas muscle compared with nonoccluded animals ($P < 0.001$). High-speed running decreased blood flows to the abdominal, psoas, and diaphragm muscles in the nonoccluded group and the psoas muscle in the acutely occluded group ($P < 0.001$).

**DISCUSSION**

The results from the present study demonstrate that exogenous administration of bFGF can induce collateral vessel remodeling in frankly hypertensive SHR (~190 mmHg aortic pressure) with occlusion of both femoral arteries. Collateral-dependent blood flows and conductances to the distal hindlimb and calf muscles were increased in a dose-dependent manner (Figs. 1 and 1). The results from the present study demonstrate that exogenous administration of bFGF can induce collateral vessel remodeling in frankly hypertensive SHR (~190 mmHg aortic pressure) with occlusion of both femoral arteries. Collateral-dependent blood flows and conductances to the distal hindlimb and calf muscles were increased in a dose-dependent manner (Figs. 1 and 1).

**Table 3. Hindlimb blood flow**

<table>
<thead>
<tr>
<th>Blood Flow</th>
<th>Nonoccluded</th>
<th>Acutely Occluded</th>
<th>0.0 Vehicle</th>
<th>0.5 Low</th>
<th>5.0 Mid</th>
<th>50.0 High</th>
<th>ANOVA Treatment Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>115 ± 13.0</td>
<td>53 ± 6.1</td>
<td>48 ± 4.7</td>
<td>65 ± 4.1</td>
<td>67 ± 3.3</td>
<td>67 ± 3.0</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>Proximal</td>
<td>119 ± 15.5</td>
<td>84 ± 9.6</td>
<td>67 ± 6.6</td>
<td>92 ± 6.3</td>
<td>91 ± 4.6</td>
<td>88 ± 4.5</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>Distal</td>
<td>110 ± 11.0</td>
<td>7 ± 0.8</td>
<td>20 ± 1.8</td>
<td>27 ± 1.4</td>
<td>31 ± 2.4</td>
<td>34 ± 1.3</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>GPS group</td>
<td>199 ± 18.7</td>
<td>10 ± 1.0</td>
<td>36 ± 3.2</td>
<td>45 ± 2.0</td>
<td>62 ± 4.6</td>
<td>62 ± 2.2</td>
<td>$P &lt; 0.001$</td>
</tr>
</tbody>
</table>

Data (in ml·min$^{-1}$·100 g$^{-1}$) are expressed as means ± SE. Significantly different from *nonoccluded control group, †acutely occluded group, ‡16 days + vehicle infusion group, and ‡‡bFGF 0.5 µg·kg$^{-1}$·day$^{-1}$ group by Tukey’s post hoc test ($P < 0.05$).
Kidney occlusion of the femoral artery is the distal muscle and 2) similar to that observed for normotensive rats (24, 46). The resistance of this collateral circuit (on the order of several centimeters). This is because preexisting arterial branches off the thigh (45). Preexisting arterial branches off the calf muscle, which is a long distance downstream from acutely occluded group; a,b,c P < 0.001

Data (in ml·min⁻¹·100 g⁻¹) are expressed as means ± SE. Significantly different from *nonoccluded control group, †acutely occluded group, ‡occluded 16 days + vehicle infusion group; and †bFGF 0.5 μg·kg⁻¹·day⁻¹ group by Tukey’s post hoc test (P < 0.05).

and 2) similar to that observed for normotensive rats (44). Although the tissue most at risk of ischemia with occlusion of the femoral artery is the distal muscle (e.g., calf muscle), the pathway for increasing collateral blood flow is via remodeling of the vessels upstream in the thigh (45). Preexisting arterial branches off bypass the occluded femoral artery and reenter the distal arteries at the level of the knee (24, 46). The resistance of this collateral circuit (Rc) that bypasses the occlusion is the primary determinant of distal hindlimb blood flow, contributing 75–85% of the total resistance to flow when resistance of the distal tissue is minimal (47). Reduction of Rc, by structural enlargement of collateral vessel diameter and/or by increase in the number of collateral vessels, enhances collagen-dependent blood flow. Although bFGF infusion increases vessel size and density of this collateral circuit (7, 12, 41–44, 46), there can also be an increase in capillarity (angiogenesis) in the adjacent thigh muscle (8). It is likely, however, that this enhanced angiogenesis is of little benefit in providing flow to the calf muscle, which is a long distance downstream (on the order of several centimeters). This is because capillaries function in a diffusive, rather than conductive, capacity in a vascular circuit; rather, it is essential to enhance the cross section of the larger conduit

Table 5. Kidney, abdominal muscle, psoas muscle, and diaphragm blood flow

<table>
<thead>
<tr>
<th></th>
<th>Nonoccluded Control</th>
<th>Acutely Occluded</th>
<th>Oceluded 16 days + bFGF, µg·kg⁻¹·day⁻¹</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 9)</td>
<td>(n = 17)</td>
<td>0.0 Vehicle</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5 Low</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>5.0 Mid</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>50.0 High</td>
<td></td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 m/min</td>
<td>532 ± 69.3</td>
<td>323 ± 36.6⁺</td>
<td>269 ± 24.1⁺</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>25 m/min</td>
<td>284 ± 51.5⁺</td>
<td>187 ± 39.8⁺</td>
<td>201 ± 21.3⁺</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td><strong>Abdominal muscle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 m/min</td>
<td>67 ± 20.4</td>
<td>77 ± 13.4</td>
<td>53 ± 9.2</td>
<td>NS</td>
</tr>
<tr>
<td>25 m/min</td>
<td>42 ± 8.9⁺</td>
<td>58 ± 14.1</td>
<td>70 ± 13.1</td>
<td></td>
</tr>
<tr>
<td><strong>Psoas Muscle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 m/min</td>
<td>224 ± 70.2</td>
<td>234 ± 34.7</td>
<td>103 ± 18.5ᵇ</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>25 m/min</td>
<td>129 ± 27.8⁺</td>
<td>180 ± 22.4⁺</td>
<td>119 ± 16.5ᵇ</td>
<td></td>
</tr>
<tr>
<td><strong>Diaphragm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 m/min</td>
<td>317 ± 85.5⁺</td>
<td>233 ± 54.6⁺</td>
<td>219 ± 24.2⁺</td>
<td>NS</td>
</tr>
<tr>
<td>25 m/min</td>
<td>240 ± 54.2⁺</td>
<td>199 ± 34.4⁺</td>
<td>256 ± 27.5⁺</td>
<td></td>
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Data (in ml·min⁻¹·day⁻¹) are expressed as means ± SE. *Significantly different from nonoccluded control group; ‡significantly different from acutely occluded group; †significantly different from 20 m/min value by Tukey’s post hoc test (P < 0.05).
vessels in the thigh to meaningfully increase flow capacity to the distant calf muscles. This places the impact of vascular remodeling by angiogenesis more on nutrient exchange in the local tissue, whereas arteriogenesis impacts flow capacity downstream. Thus we interpret our results to indicate that the increase in collateral blood flow is due to enlargement of preexisting arteries that function as collateral conduits, as a result of the vascular remodeling described by Schaper and coworkers (21).

We measured blood flow during treadmill exercise to assess changes in the maximal capacity of the collateral circuit when blood flow to the calf muscle is determined by $R_c$. This is fundamentally different from the collateral assessment obtained from limb flow recovery, relative to the “resting” flow of the contralateral nonoccluded limb, by using laser-Doppler technology (32). Determining collateral flow capacity requires that the distal vascular resistance within the calf muscles becomes a relatively small fraction of the total resistance in the circuit. This was achieved by running the rats at a challenging speed, which in the nonoccluded SHR resulted in a calf muscle blood flow of ~200 ml·min$^{-1}$·100 g$^{-1}$ (cf. Table 3). An expected similar flow demand by the calf muscles in the occluded SHR was caused by the same exercise; however, calf blood flow was only 5–30% as great, depending on treatment group (Table 3). Thus flow was limited by the upstream $R_c$. A second blood flow measurement was then made, but at a higher running speed that should have reduced calf muscle resistance even further, if possible. Then, when the flow measures at the two running speeds were similar, we interpreted the values to represent maximal collateral-dependent blood flow (43, 44, 48). It is possible, however, that the flows determined in this manner are confounded by vasodilatory responses that do not necessarily reflect structural changes in the collateral vessels in the thigh. For example, previous evidence demonstrates that these thigh vessels that function as collaterals are vasoreactive (39, 40, 45, 47). However, the change is modest (~15%), operating within the existing vessel caliber. The increase in collateral blood flow with bFGF measured in this study is well in excess of the acute vasodilatory response indicating the presence of collateral artery enlargement typical of that obtained in nonhypertensive rats (42–44, 46). Furthermore, we believe that our findings are not confounded by the acute vasodilatory response that bFGF can cause (13). First, the minimal dose of bFGF needed to impart a dilatory response is well above the infusion rate (~45-fold higher) of even the high dose that we used in this study. Second, collateral blood flow was determined on day 16, well after the 14-day osmotic pump culminates normal bFGF delivery. Interestingly, the high-dose SHR group exhibited a significant decrease in blood pressure but the same absolute increase in collateral blood flow. This tends to support the importance of the structural changes in the collateral circuit.

We previously reported (42, 44, 46) that exogenous bFGF administration induces collateral vessel remodeling and improves collateral blood flow in rats with normal blood pressure. This is similar to numerous other studies showing the efficacy of bFGF (7, 12). Therefore, we did not believe that it was essential to replicate this response in the normotensive control rats that are often used as control for SHR (e.g., Kyoto rats). Rather, we simply determined whether an altered endothelial responsiveness, observed in these frankly hypertensive SHR (22, 26–28, 30), would preempt an improvement in collateral blood flow following occlusion of a major peripheral vessel, as appears to be the case with an absolute loss of eNOS in the mouse (32). Although the response of SHR to exogenous bFGF administration appears similar to that observed in normotensive rats, there may be differences. For example, it is not known whether the rarefaction phenomenon found in hypertensive animals (18, 19, 34) and humans (2, 20) could be counteracted by exogenous bFGF. The actions of this cytokine and the modest reduction in blood pressure suggest this possibility, if high doses are used.

Role of eNOS-NO and bFGF in collateral vascular remodeling. Exogenous delivery of bFGF infusion can cause vascular dilation and a hypotensive reaction by bFGF-stimulated NO release (13). In the present study, a lower systemic blood pressure was found in...
the high-dose bFGF group (Table 2). As discussed above, we believe that this lower blood pressure is not due to an acute effect of bFGF; rather, we hypothesize that it is due to an increase in eNOS activity induced by the chronic administration of bFGF. bFGF is known to upregulate eNOS mRNA expression (6), and increased NO bioavailability lowers blood pressure in the SHR (1). Furthermore, Cuevas et al. (14) found that SHR exhibit decreased expression of PGF and eNOS, and when the endothelial contents of FGF and eNOS were returned to normal, blood pressure was similarly reduced to normal. Thus the bFGF-induced reduction of the systemic blood pressure observed at the high dose in the SHR could be due to restored NO bioavailability and endothelium-dependent vasodilation. Experimental support for this hypothesis could also be important in underpinning the bFGF-induced increase in collateral blood flow, because we previously showed (29) that normal NOS activity is essential for bFGF-induced arteriogenesis.

Cardiovascular responses to femoral artery occlusion. Neither acute nor chronic occlusion of both femoral arteries caused noticeable changes of blood pressure and heart rate in the SHR (Table 2). There was no sign of any ischemia-related damage of the affected hindlimbs. Furthermore, during treadmill running, we did not observe the pressor response attributed to contractions of ischemic muscle of SHR, as described in dogs (35) and rats (43). However, we did find that blood flows to the kidney were lower in all groups with femoral artery occlusion during exercise (Table 5). This may indicate an increased output of sympathetic nerve activity that was significant enough to cause vasoconstriction of the kidney, but not enough to raise the already elevated systemic blood pressure. We did not observe any other signs of functional cardiovascular disturbance after bilateral occlusion of the femoral artery.

Clinical implications. Hypertension is a major risk factor for PAD, with a much higher age-adjusted risk in men (2.5-fold) and women (3.9-fold) to develop PAD (25). In addition, PAD patients exhibit a high rate of hypertension (83–88% in men and women; Ref. 33) and suffer higher incidents of coronary heart disease or cerebral vascular disease (4). Unfortunately, there are limited noninvasive options for treatment of PAD patients, especially for those who do not qualify for vascular reconstructive surgery. Therapeutic angiogenesis offers a potential avenue for treating those patients. Our study provides additional information demonstrating that exogenous bFGF enhances collateral blood flow in spontaneously hypertensive animals. If these observations are generally applicable, then the presence of hypertension does not preempt possible therapeutic angiogenesis, contraindications notwithstanding.

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DISCLOSURES

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REFERENCES


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