Responsiveness of Iliac Collateral Vessels to Constrictor Stimuli in Atherosclerotic Primates

J. Antonio G. Lopez, Mark L. Armstrong, David G. Harrison, Donald J. Piegors, and Donald D. Heistad

This study was performed to examine, first, the protective effects and responses of collateral vessels of the hind limb in normal and atherosclerotic monkeys and, second, the effects of chronic arterial occlusion on the development of atherosclerosis. The iliac artery was ligated on one side in cynomolgus monkeys. Sixteen months later, we recorded the pressure gradient across the limb collaterals and measured blood flow with microspheres. Collateral conductance was fivefold greater after chronic ligation of the iliac artery than after acute ligation. Despite dilatation or growth of collateral vessels after chronic ligation, iliac pressure was reduced distal to the ligation. Blood flow to the limb was normal after chronic ligation in both normal and atherosclerotic monkeys. Collateral vessels constricted in response to infusion of phenylephrine and serotonin in normal and atherosclerotic monkeys. Thus, one conclusion of this study is that collateral vessels restore limb blood flow to normal after chronic vascular occlusion in both normal and atherosclerotic monkeys, but the protective effects of collateral vessels may be compromised by vasoconstrictor stimuli. Morphometric measurements indicated that occlusion of the iliac artery reduced proliferation of atherosclerotic intima distal to the occlusion in the cholesterol-fed monkeys. Thus, a second conclusion of this study is that atherosclerosis is attenuated below an arterial occlusion. (Circulation Research 1988;63:1020-1028)

The collateral circulation of the limb is an alternative pathway for blood flow when a major blood vessel is occluded. After arterial occlusion, blood flow distal to the occlusion returns rapidly toward normal, which suggests that preexistent collateral vessels open or dilate. During subsequent weeks, there is an increase in the number and size of collateral vessels. All previous studies of collateral circulation have been conducted in normal preparations. Furthermore, there have been no studies of collateral vessels in the limb more than a few weeks after arterial occlusion.

Atherosclerosis may produce an occlusive lesion and thus lead to the formation or growth of collateral vessels. In the presence of vascular occlusion, distal perfusion depends on the opening, development, and reactivity of collateral vessels. Atherosclerosis might promote the development of collateral vessels through the release of growth factors or inhibit development of collaterals because of endothelial dysfunction.

Recent evidence suggests that large collateral vessels, which are present within two weeks after ligation of the superficial femoral artery, are very responsive to serotonin. Vasoactive effects of serotonin are of interest because platelets, which contain large amounts of serotonin, may aggregate at the site of atherosclerotic lesions and release serotonin. Thus, if platelets aggregate in the aorta or other arteries, high levels of serotonin may occur in arterial blood downstream and might affect collateral vessels. Thus, the first goal of these experiments was to test the hypothesis that vasoactive stimuli may compromise the protective effect of collateral vessels. We examined responses to stimulation of serotonergic and adrenergic receptors. Unique features of these studies were that responses were examined in both normal and atherosclerotic primates, and the studies were performed more than a year after arterial occlusion.
Hypertension increases the development of atherosclerosis, and epidemiological studies suggest that low levels of pressure attenuate the development of atherosclerosis. Experimental studies suggest that attenuation of hypertension below coarctation of the thoracic aorta reduces development of atherosclerosis. A clinical observation is that progression of atherosclerosis in the limb appears to be attenuated distal to a chronic occlusion. The authors speculated that reduction of arterial pressure or blood flow, even below normal levels, may attenuate the development of atherosclerosis. The second goal of these experiments was to test the hypothesis that reduction of pressure in limb arteries of normotensive primates attenuates the development of atherosclerosis.

Materials and Methods

Two groups of male Malaysian cynomolgus monkeys were studied. Eleven normal monkeys were fed commercial laboratory chow (Purina Monkey Chow, Ralston Purina, Richmond, Indiana). Twelve monkeys were fed atherogenic diet, which contained cholesterol, 1 mg/calorie, and fat, 41% of total calories, for 18–20 months. The normal monkeys weighed 5.8±0.3 kg (mean±SEM) and the atherosclerotic group weighed 5.3±0.2 kg. At intervals of 3–4 months, the monkeys were sedated with ketamine hydrochloride (10 mg/kg i.m.), and a venous blood sample was obtained. Total cholesterol and triglycerides were determined with the method used by the Lipid Research Clinics Protocol. The monkeys were fed an atherogenic diet, which contained cholesterol, 1 mg/calorie, and fat, 41% of total calories, for 18–20 months. The normal monkeys weighed 5.8±0.3 kg (mean±SEM) and the atherosclerotic group weighed 5.3±0.2 kg. At intervals of 3–4 months, the monkeys were sedated with ketamine hydrochloride (10 mg/kg i.m.), and a venous blood sample was obtained. Total cholesterol and triglycerides were determined with the method used by the Lipid Research Clinics Protocol.

Ligation of Left Common Iliac Artery

The monkeys were sedated with ketamine hydrochloride (30 mg/kg i.m.) and acepromazine maleate (1 mg/kg i.m.). Local anesthesia was achieved by infiltration with 2% lidocaine hydrochloride. A 6 cm laparotomy was made with aseptic technique. The bifurcation of the aorta was exposed, and the left common iliac artery was ligated approximately 0.5–1.0 cm below the bifurcation. One normal monkey developed signs of tissue ischemia several days after surgery and was anesthetized and euthanized. All other monkeys were active and appeared to be healthy after surgery.

Measurement of Collateral Hemodynamics

Approximately 16 months after ligation of the iliac artery (15.8±0.3 months in normal and 15.6±0.2 months in atherosclerotic monkeys), the monkeys were sedated with ketamine (15 mg/kg i.m.) and anesthetized with chloralose (100 mg/kg i.v.). A tracheostomy was performed, and the monkeys were intubated and ventilated with room air and supplemental oxygen. Gallamine triethiodide (5 mg/kg i.v.) was given for paralysis of skeletal muscles and heparin sodium (500 units/kg i.v.) was given for anticoagulation. Arterial blood gases and pH were monitored during each study and maintained at normal levels by adjustment of the ventilatory rate, flow rate of oxygen or injection of small amounts of sodium bicarbonate. Rectal temperature was maintained at 37°–38° C with a heating pad.

A polyethylene catheter was inserted through a brachial artery for measurement of aortic pressure and to obtain blood samples. Catheters were placed into a brachial and carotid artery for reference blood samples. Catheters were inserted into the brachial veins for injections of fluids and drugs.

Pressure was measured with a Jelco 24 gauge catheter (Critikon, Tampa, Florida) in both common iliac arteries at the origin of the internal iliac artery. Pressure in both dorsal pedal arteries was measured by retrograde cannulation with PE50 tubing.

Measurement of Hind Limb Blood Flow With Microspheres

Through a thoracotomy, two catheters were inserted into the left atrium: one was used for injection of microspheres and the other for infusion of serotonin. Total and regional blood flow to the hind limbs was determined by using microspheres 15 μm mean diameter labeled with 52Sc, 85Sr, 95Nb, 113Sr, 114Ce, and 153Gd (New England Nuclear, Boston, Massachusetts). Microspheres were injected into the left atrium in 15 seconds. Reference blood samples were withdrawn at 1.3 ml/min from a brachial and carotid artery for 10 seconds before injection of microspheres until 2 minutes after injection of microspheres.

The monkey was killed with intravenous potassium chloride at the end of the experiment. Both hind limbs were removed and sectioned into muscle, skin, and bone and counted separately. These values were pooled to obtain flow to the limb. Both the tissue and reference blood samples were counted for 3–5 minutes on a gamma counter. The isotope separation was performed by standard techniques. Total blood flow to the limb (TBF) was calculated from the equation: TBF= (counts/g limb × 100 × withdrawal rate of reference blood samples) / counts in the reference blood samples.

In five monkeys, arteriovenous shunting of 15 μm microspheres in the limb was examined. A PE90 catheter was advanced from a distal segment of the femoral vein to the bifurcation of the common iliac vein. During injection of microspheres into the left atrium, blood samples from the iliac vein were collected at a flow rate equal to arterial reference samples (1.3 ml/min). Shunting was calculated from the difference in radioactivity of arterial and venous blood samples during control, acute occlusion of the iliac artery, and infusion of serotonin and phenylephrine. Percent shunt flow was calculated from the radioactivity in the venous blood samples × 100/ radioactivity in the arterial blood samples. Shunting of microspheres in the limb during all interventions was less than one percent.
**Experimental Protocol**

Measurements of limb blood flow and pressure were obtained during the control period (infusion of saline), during occlusion of the right common iliac artery for 5 minutes, during infusion of serotonin into the left atrium, during a second control period (infusion of saline), and during intravenous phenylephrine. We infused 10 \(\mu g/kg\min\) of serotonin (5-hydroxytryptamine creatine sulfate complex; Sigma Chemical, St. Louis, Missouri) into the left atrium and 25 \(\mu g/kg\min\) of L-phenylephrine hydrochloride (Sigma Chemical, St. Louis, Missouri) intravenously. Serotonin was administered into the left atrium because it is removed rapidly by the pulmonary circulation when given intravenously.\(^{26}\) Saline (vehicle) was infused at 0.58 ml/min intravenously or into the left atrium. Phenylephrine tended to increase arterial pressure in both normal and atherosclerotic monkeys, and serotonin tended to increase arterial pressure in atherosclerotic monkeys. Blood was removed during infusion of phenylephrine in normal and atherosclerotic monkeys and during infusion of serotonin in atherosclerotic monkeys to prevent an increase in arterial pressure.

Serotonin or phenylephrine was infused for about 4 minutes before microspheres were injected, and the infusions were continued for 2 minutes after injection of microspheres. We waited at least 20 minutes between interventions.

**Morphological Studies**

At the end of the study, the abdominal aorta was cannulated antegradely at the level of the kidneys, and the limb vessels were perfused for 2–3 minutes with approximately 300 ml or normal saline solution containing adenosine 1 mg/ml and papaverine 0.25 mg/ml. Barium was then used to fill the limb vessels at a pressure of 100 mm Hg.

The iliac and femoral arteries and visible collateral vessels were removed, examined for gross atherosclerotic lesions, and fixed in 10% buffered formalin. Histological study was carried out on paraffin-embedded sections. Sections were stained with hematoxylin-eosin and Verhoeff-Van Gieson. Morphometric determination of the size of the intima and media was performed with an image analyzer, as described previously.\(^{26}\)

**Data Interpretation and Statistical Analysis**

Total limb conductance was determined by dividing the blood flow to the limb by the pressure in the iliac artery. Large artery conductance was calculated by dividing blood flow to the limb by the pressure gradient from the iliac artery to the dorsal pedal artery. Collateral conductance was determined by dividing blood flow to the limb by the pressure gradient from the aorta to the iliac artery.

Values were compared for interventions and between groups with a nested factorial analysis of variance. Student's \(t\) test was used for morphometric comparisons. Bonferroni correction was used when multiple comparisons were made.\(^{27}\) A significance level of \(\alpha=0.05\) was used. Values are expressed as mean±SEM.

**Results**

**Plasma Lipids**

Plasma cholesterol was 112±22 mg/dl in normal monkeys and 658±93 mg/dl during the last 3 months of atherogenic diet in atherosclerotic monkeys. Plasma triglycerides were <40 mg/dl in normal and atherosclerotic monkeys.

**Morphologic Findings**

Normal monkeys had no gross lesions in the limb arteries. After 18–20 months of atherogenic diet, there was a well defined histological picture of fibrofatty intimal thickening with areas of necrosis and calcification in the iliac and femoral arteries.\(^{26}\) There appeared to be less necrosis and calcification in vessels of the ligated limb than in vessels of the intact limb.

Collateral vessels were approximately 200 to 900 \(\mu m\) in diameter and appeared free of atherosclerotic lesions in both control and atherosclerotic monkeys. Several layers of smooth muscle were seen on histological examination. Most collaterals that were identified arose from the spinal and caudalis arteries or from one of their branches. These vessels joined the proximal internal or external iliac artery.

**Morphometry**

After 18–20 months of atherogenic diet, there was marked intimal thickening in the iliac and femoral arteries (Table 1). Medial mass was similar in normal and atherosclerotic monkeys. The lumen tended to be larger in atherosclerotic monkeys than in normal monkeys, as described previously.\(^{26}\)

In atherosclerotic monkeys, intimal mass in the iliac and proximal femoral artery was significantly less in the ligated limb than in the intact limb (Table 1, Figure 1). In normal and atherosclerotic monkeys, medial mass tended to be less in the ligated limb than in the contralateral limb, but there was no consistent difference.

**Hemodynamic Responses to Acute Occlusion of the Iliac Artery**

In normal monkeys, acute occlusion of the previously intact right iliac artery produced a marked decrease in iliac pressure and total limb flow (Figure 2). In contrast, in the left limb, in which the iliac artery was chronically occluded, iliac pressure returned toward normal and total limb flow was restored to normal (Figure 2). After acute occlusion of the iliac artery in normal monkeys, collateral conductance was low. There was a fivefold increase...
TABLE 1. Effects of Ligation of Iliac Artery on Arterial Wall Mass and Lumen Size in Normal and Atherosclerotic Monkeys

<table>
<thead>
<tr>
<th>Artery</th>
<th>Location</th>
<th>Normal</th>
<th>Atherosclerotic</th>
<th>Normal</th>
<th>Atherosclerotic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intact limb</td>
<td></td>
<td>Ligated limb</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal</td>
<td>Atherosclerotic</td>
<td>Normal</td>
<td>Atherosclerotic</td>
</tr>
<tr>
<td>Iliac artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intima</td>
<td>Proximal</td>
<td>0.03±0.03</td>
<td>0.85±0.26*</td>
<td>&lt;0.01</td>
<td>0.36±0.13*</td>
</tr>
<tr>
<td>Media</td>
<td>Proximal</td>
<td>0.49±0.07</td>
<td>0.69±0.27</td>
<td>0.37±0.05</td>
<td>0.42±0.08</td>
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<tr>
<td>Lumen</td>
<td>Proximal</td>
<td>2.93±0.71</td>
<td>3.39±0.98</td>
<td>3.07±1.01</td>
<td>2.89±0.78</td>
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<tr>
<td>Femoral artery</td>
<td>Proximal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intima</td>
<td>Mid-portion</td>
<td>0.03±0.03</td>
<td>1.23±0.23*</td>
<td>0.02±0.02</td>
<td>0.74±0.20*</td>
</tr>
<tr>
<td>Media</td>
<td>Mid-portion</td>
<td>1.12±0.43</td>
<td>0.73±0.11</td>
<td>0.64±0.07</td>
<td>0.68±0.06</td>
</tr>
<tr>
<td>Lumen</td>
<td>Mid-portion</td>
<td>2.85±0.68</td>
<td>4.33±0.77</td>
<td>2.52±0.95</td>
<td>3.92±0.58</td>
</tr>
<tr>
<td>Mid-portion</td>
<td></td>
<td>&lt;0.01</td>
<td>0.38±0.13*</td>
<td>&lt;0.01</td>
<td>0.27±0.11*</td>
</tr>
<tr>
<td>Media</td>
<td></td>
<td>0.56±0.12</td>
<td>0.60±0.06</td>
<td>0.43±0.04</td>
<td>0.46±0.03*</td>
</tr>
<tr>
<td>Lumen</td>
<td></td>
<td>1.04±0.25</td>
<td>2.40±0.40*</td>
<td>1.18±0.28</td>
<td>2.02±0.27*</td>
</tr>
</tbody>
</table>

Values are area (in mm²) in 11 normal and 12 atherosclerotic monkeys. Values in ligated limb are distal to the ligation, and values in intact limb were obtained at the same level of each artery.

Values are mean±SEM. *p<0.05 vs. normal.
†p<0.05 vs. contralateral intact limb.

in collateral conductance after chronic occlusion of the iliac artery (Figure 2).

In atherosclerotic monkeys, responses to acute and chronic occlusion were similar to responses in normal monkeys (Figure 3). Acute occlusion reduced iliac pressure and limb blood flow, and after chronic occlusion, iliac pressure and limb blood flow returned toward normal. In atherosclerotic monkeys, there was a sixfold increase in collateral conductance after chronic occlusion of the iliac artery (Figure 3).

Responses to Phenylephrine

In the intact limb of normal monkeys, phenylephrine decreased blood flow to the limb. Phenylephrine decreased conductance of large arteries in the limb and thus reduced distal pressure in the dorsal pedal artery (Table 2, Figure 4).

In the ligated limb, the baseline (control) iliac pressure and dorsal pedal pressure were lower than in the intact limb (Table 2). Phenylephrine reduced flow to the limb and decreased iliac pressure. Phenylephrine produced a significant decrease in collateral conductance and also decreased conductance of large arteries in the limb.

In atherosclerotic monkeys, responses to phenylephrine were similar to those in normal monkeys (Table 2, Figure 4). Phenylephrine decreased conductance of large arteries in both the intact and ligated limbs. Phenylephrine produced a significant decrease in conductance of collateral vessels.

FIGURE 1. Proximal femoral artery from the intact limb (left) and chronically occluded limb (right) of an atherosclerotic monkey. There is less intimal proliferation in the chronically occluded limb; Verhoeff-Van Gieson stain, magnification, ×24.
NORMAL MONKEYS

Responses to Serotonin

In the intact limb of normal monkeys, blood flow to the limb and total limb conductance were unchanged during infusion of serotonin. Serotonin decreased conductance of large arteries (Table 3, Figure 5).

In the ligated limb of normal monkeys, serotonin did not decrease large artery conductance. Serotonin reduced collateral conductance.

In the intact limb of atherosclerotic monkeys, serotonin tended to reduce flow to the limb and produced a marked decrease in conductance of large arteries (Table 3, Figure 5). In the ligated limb of atherosclerotic monkeys, serotonin did not significantly reduce conductance of large arteries. Serotonin reduced collateral conductance and blood flow to the limb.

Discussion

These experiments demonstrate responses of hind limb collateral vessels more than one year after occlusion of the common iliac artery in normal and atherosclerotic cynomolgus monkeys. In response to chronic iliac occlusion, there was a marked increase in collateral conductance that was sufficient to normalize blood flow to the limb in both normal and atherosclerotic monkeys. The smooth muscle of the collateral vessels constricted in response to phenylephrine and serotonin in both normal and atherosclerotic monkeys, which compromised collateral perfusion.

In relation to development of atherosclerosis, the major new finding was that occlusion of the common iliac artery inhibited the development of atherosclerosis in the hind limb arteries. Thus, reduction of pressure in the artery attenuated the development of atherosclerosis even in normotensive monkeys.

Advantages and Limitations of Preparation

The preparation provided a sensitive means for detection of vascular responses of collateral vessels and native large arteries in vivo. This approach allowed measurement of transcollateral pressure and the pressure gradient of large arteries within the limb. By measuring blood flow to the limb, we were able to calculate collateral conductance and conductance of large arteries of the limb.

It would have been desirable to infuse vasoconstrictor stimuli directly into collateral vessels to avoid systemic responses. The origin of all collateral vessels to the limb could not be determined, however. Therefore, drugs were infused systemi-

ATHEROSCLEROTIC MONKEYS

Responses to Serotonin

In the intact limb of atherosclerotic monkeys, blood flow to the limb and total limb conductance were unchanged during infusion of serotonin. Serotonin decreased conductance of large arteries (Table 3, Figure 5).

In the ligated limb of atherosclerotic monkeys, serotonin did not decrease large artery conductance. Serotonin reduced collateral conductance.

In the intact limb of atherosclerotic monkeys, serotonin tended to reduce flow to the limb and produced a marked decrease in conductance of large arteries (Table 3, Figure 5). In the ligated limb of atherosclerotic monkeys, serotonin did not significantly reduce conductance of large arteries. Serotonin reduced collateral conductance and blood flow to the limb.
TABLE 2. Effect of Phenylephrine in Normal and Atherosclerotic Monkeys

<table>
<thead>
<tr>
<th></th>
<th>Intact limb</th>
<th>Ligated limb</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Phenylephrine</td>
</tr>
<tr>
<td>Normal monkeys (n=11)</td>
<td></td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iliac</td>
<td>84±4</td>
<td>83±4</td>
</tr>
<tr>
<td>Dorsal pedal</td>
<td>71±4</td>
<td>56±6*</td>
</tr>
<tr>
<td>Total limb Conductance (ml/min/mm Hg/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large artery</td>
<td>4.5±0.8</td>
<td>2.0±0.2*</td>
</tr>
<tr>
<td>Collateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atherosclerotic monkeys (n=12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iliac</td>
<td>88±5</td>
<td>85±6</td>
</tr>
<tr>
<td>Dorsal pedal</td>
<td>67±5</td>
<td>55±6</td>
</tr>
<tr>
<td>Total limb Conductance (ml/min/mm Hg/g)</td>
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<tr>
<td>Large artery</td>
<td>4.6±0.8</td>
<td>2.2±0.3*</td>
</tr>
<tr>
<td>Collateral</td>
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<td></td>
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</table>

Values are mean±SEM. Phenylephrine was infused at 25 μg/kg/min i.v.  
*p<0.05 vs. control values in ipsilateral limb.  
†p<0.05 vs contralateral limb.  
No values were significantly different in normal versus atherosclerotic monkeys.

Previous Studies of Limb Collateral Arteries

There have been several studies of vasoconstrictor responses in limb collateral vessels. Responses of collateral vessels have not been studied in nonhuman primates, in cholesterol-fed animals, or more than 3 weeks after occlusion of an artery in the limb.

Thulesius studied cats 2 weeks after occlusion of the femoral artery. Blood flow was determined by a photoelectric drop counter that measured venous outflow from the femoral vein. Collateral conductance was greater after chronic occlusion than after acute occlusion, but the data were descriptive and not quantitative.

Coffman studied responses in dogs 3 weeks after ligation of the external iliac artery. Intra-aortic infusions of norepinephrine and epinephrine produced no change in collateral resistance. In contrast, intravenous norepinephrine resulted in a decrease in collateral resistance, which probably was due to a reflex mechanism secondary to an increase in systemic arterial pressure. Interventions that decreased systemic arterial pressure produced constrictor responses of collateral vessels after acute, but not chronic, occlusion.

Helical strips from collateral vessels in dogs, after ligation of the external iliac artery. Intra-aortic infusions of norepinephrine and epinephrine produced no change in collateral resistance. In contrast, intravenous norepinephrine resulted in a decrease in collateral resistance, which probably was due to a reflex mechanism secondary to an increase in systemic arterial pressure. Interventions that decreased systemic arterial pressure produced constrictor responses of collateral vessels after acute, but not chronic, occlusion.

Helical strips from collateral vessels in dogs, after ligation of the superficial femoral artery, were more responsive than native arteries of similar size to α-adrenergic agonists. This finding contrasts with responses of mature coronary collateral vessels in intact hearts and isolated rings of coronary collaterals, in which responses to α-adrenergic agonists could not be demonstrated.

![Figure 4. Response to infusion of phenylephrine in the chronically occluded limb in normal and atherosclerotic cynomolgus monkeys. Values are mean±SEM in 11 normal and 12 atherosclerotic monkeys, during control (Con) and infusion of phenylephrine (Phenyl); *p<0.05 versus control.](image-url)
TABLE 3. Effect of Serotonin in Normal and Atherosclerotic Monkeys

<table>
<thead>
<tr>
<th></th>
<th>Intact limb</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Serotonin</td>
<td>Control</td>
<td>Serotonin</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
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<tr>
<td>Iliac</td>
<td>79±4</td>
<td>77±5</td>
<td>58±6†</td>
<td>46±8†</td>
</tr>
<tr>
<td>Dorsal pedal</td>
<td>66±4</td>
<td>55±5</td>
<td>46±6†</td>
<td>35±6†</td>
</tr>
<tr>
<td>Conductance (ml/min/mm Hg/g)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total limb</td>
<td>4.9±1.0</td>
<td>4.8±0.8</td>
<td>4.1±0.7</td>
<td>3.5±0.4</td>
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<td>Large artery</td>
<td>31±9.3</td>
<td>18±2.4*</td>
<td>35±8.1</td>
<td>29±5.4</td>
</tr>
<tr>
<td>Collateral</td>
<td>. . .</td>
<td>. . .</td>
<td>15±1.5</td>
<td>10±1.9*</td>
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<td>Atherosclerotic monkeys (n=9)</td>
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<td>Mean arterial pressure (mm Hg)</td>
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<tr>
<td>Iliac</td>
<td>82±5</td>
<td>82±5</td>
<td>63±5†</td>
<td>46±5*†</td>
</tr>
<tr>
<td>Dorsal pedal</td>
<td>61±7</td>
<td>24±5††</td>
<td>41±5†</td>
<td>16±2*††</td>
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<td>Conductance (ml/min/mm Hg/g)</td>
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</tr>
<tr>
<td>Total limb</td>
<td>4.0±0.6</td>
<td>2.6±0.4*†</td>
<td>4.6±1.1</td>
<td>2.2±0.3*</td>
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<tr>
<td>Large artery</td>
<td>19±3.9</td>
<td>4.6±1.1*</td>
<td>18±3.9</td>
<td>12±1.8†</td>
</tr>
<tr>
<td>Collateral</td>
<td>. . .</td>
<td>. . .</td>
<td>15±2.7</td>
<td>6.3±1.8*</td>
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</table>

Values are mean±SEM. Serotonin was infused at 10 μg/kg/min.
*p<0.05 vs. control values.
†p<0.05 vs. control contralateral limb.
‡p<0.05 vs. normal monkeys.

Responses to Serotonin and Phenylephrine

Serotonin produces only modest constriction of large arteries in normal dogs31 and monkeys.32 Atherosclerosis greatly potentiates vasoconstrictor responses to serotonergic stimuli in vitro33 and in vivo.32 Platelets, which contain large amounts of serotonin,18 may aggregate at the site of atherosclerotic lesions and release serotonin,19 so that very high levels of serotonin occur in the vessel wall beneath a thrombus.20 Orlandi et al17 suggested, based on arteriographic studies, that limb collateral vessels of the dog are very sensitive to serotonin. They observed that norepinephrine did not constrict visible collateral vessels.

We have estimated the blood levels of serotonin that were achieved in this study and compared them with levels that have been observed in the presence of thrombi in vivo. We assumed that 90% of serotonin is cleared in one passage through the pulmonary circulation,35 that the circulation time in monkeys is 14 seconds,34 and that the volume of distribution is limited to plasma. We thereby estimated35 that infusion of serotonin at 10 μg/kg/min into the left atrium in this study produced plasma concentrations of 43 ng/ml. Thus, the dose of serotonin used in this study appears to produce plasma levels of serotonin that are lower than those that have been measured downstream from an experimental platelet thrombus (213±63 ng/ml).20

We have considered the possibility that the rate of clearance of serotonin may differ in normal and atherosclerotic monkeys. The primary sites for removal of serotonin are endothelial cells of small pulmonary vessels.36 Atherosclerotic lesions are not present in these vessels in monkeys that are fed an atherogenic diet. It seems unlikely that metabolism of serotonin is altered substantially in atherosclerotic monkeys. Serotonin also is taken up and stored by platelets. The half-life of serotonin in blood is approximately 60 to 120 seconds23 compared with 7 seconds in vivo.25-26,34 Furthermore, during bolus transit through the lung, uptake of serotonin by platelets is negligible.37 Thus, uptake of serotonin by platelets is much less important than metabolism by endothelial cells, and it is unlikely that possible differences in uptake of serotonin by platelets had major effects on vascular responses observed in our study.
Subhuman primates develop atherosclerotic lesions in large arteries that closely resemble those that occur in humans. There are marked increases in intimal area of large arteries and impaired maximal vasodilator responses in the hind limb. Limb collateral arteries did not develop atherosclerotic lesions after 15–16 months of diet, presumably because collateral vessels are only 200–900 μm in diameter, and atherosclerotic lesions generally are confined to large arteries. Constrictor responses of collateral vessels to serotonin were similar in atherosclerotic and in normal monkeys. In contrast, in native vessels of the limb, which developed marked atherosclerotic lesions, constrictor responses to serotonin were significantly greater in atherosclerotic than in normal monkeys. Thus, vessels without atherosclerosis, even if exposed to hypercholesterolemia for prolonged periods, do not develop hyperresponsiveness to serotonin.

These observations may have important clinical implications. When the limb is dependent on collateral circulation after occlusion of a major artery, it may be particularly susceptible to further compromise of blood flow. First, catecholamines may constrict collateral vessels and reduce flow to the limb. Second, if platelets adhere to atherosclerotic plaques in the aorta, aggregate, and release serotonin into the arterial blood, collateral vessels may constrict and reduce perfusion of the limb. Thus, although collateral vessels restore blood flow to the limb to normal levels under baseline conditions, constrictor stimuli may compromise the effectiveness of collateral vessels.

**Effects of Chronic Occlusion in Atherosclerosis**

We have considered mechanisms that may account for our finding that ligation of the iliac artery attenuates development of atherosclerosis in the limb. First, changes in flow may affect development of atherosclerosis. Butterfield et al produced a fistula between the external iliac artery and iliac vein in miniature swine that were fed an atherogenic diet to increase blood velocity and flow in the iliac artery. Elevation of blood flow was associated with attenuation of atherosclerosis. In our study, blood flow was similar in both limbs, and it is unlikely that changes in blood flow played a major role in attenuation of development of atherosclerosis. We cannot exclude the possibility, however, that flow to the two limbs may differ when the monkey is active. Second, reduction in pressure may attenuate the development of atherosclerosis. Attenuation of hypertension below coarctation of the thoracic aorta reduces the development of atherosclerosis. The findings in our study extend those of a previous study and suggest that reduction of arterial pressure, even in a normotensive animal, decreases the development of atherosclerosis. Third, arterial wall motion may affect the rate of development of atherosclerosis. Based on previous findings, it seems likely that reduction of arterial wall motion may contribute to the finding in this study that development of atherosclerosis is attenuated distal to arterial occlusion.

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**References**


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