Dietary Treatment of Atherosclerosis Abolishes Hyperresponsiveness of Retinal Blood Vessels to Serotonin in Monkeys

Frank M. Faraci, PhD; Mark L. Armstrong, MD; and Donald D. Heistad, MD

**Background and Purpose:** Atherosclerosis alters vascular responses of the eye to serotonin. Augmented vasoconstrictor responses in the retina to serotonin are associated with functional impairment and may contribute to amaurosis fugax. The goal of this study was to test the hypothesis that dietary treatment of atherosclerosis restores vascular responses of the eye toward normal.

**Methods:** We measured blood flow to the retina using microspheres in six normal monkeys, five atherosclerotic monkeys, and five atherosclerotic monkeys that were fed a normal (regression) diet for 18 months.

**Results:** Infusion of 8 and 40 \( \mu \text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \) serotonin into the left atrium had little effect on blood flow to the retina in normal monkeys. In contrast, the high dose of serotonin reduced blood flow to the retina by a mean±SEM of 81±9% \( (p<0.05) \) in atherosclerotic monkeys. In monkeys that were fed the regression diet, serotonin had no effect on blood flow to the retina.

**Conclusions:** Regression of atherosclerosis abolishes augmented responses of the retinal circulation to serotonin. *(Stroke 1991;22:1405-1408)*

Atherosclerosis potentiates vasoconstrictor responses to products released by platelets, including serotonin and thromboxane. Altered vascular responses in the coronary circulation to platelet products (especially serotonin) during atherosclerosis have received considerable attention in relation to the pathophysiology of angina. There is marked potentiation of constrictor responses of the coronary arteries to serotonin in humans with atherosclerosis. Atherosclerosis also impairs the basal and agonist-induced production of endothelium-derived relaxing factor (EDRF) in human coronary arteries. Such vascular abnormalities may contribute to the increased incidence of vasospasm during atherosclerosis.

We have demonstrated recently that atherosclerosis greatly potentiates vasoconstrictor responses of the retina to serotonin. This finding led us to suggest that altered responses to products released by platelets during aggregation may contribute to the pathogenesis of amaurosis fugax. Platelets may produce transient ischemia of the retina by embolization and by vasoconstriction.

There has been considerable clinical interest recently in the effects of treatment of hypercholesterolemia. Dietary treatment of atherosclerosis reduces the size of intimal lesions. Maximal vasodilatation improves in the cerebrum but not in other vascular beds. There is also improvement in endothelium-dependent responses, and augmented responses of the hind limb and cerebrum to serotonin are restored toward normal. The goal of this study was to test the hypothesis that dietary treatment of atherosclerosis abolishes hyperresponsiveness of the retinal circulation to serotonin.

**Materials and Methods**

We studied three groups of adult cynomolgus monkeys. Six normal monkeys were fed commercial chow (Purina monkey chow,Ralston Purina, Richmond, Ind.), which produced a mean±SEM plasma cholesterol concentration of 139±17 mg/dl. In a second group of five monkeys, atherosclerosis was induced by feeding an atherogenic diet that contained 41% of total calories from fat and 0.8% cholesterol for 18 months. The plasma cholesterol
concentration in this group was 624 ± 86 mg/dl. A third group of five monkeys was fed the atherogenic diet for 18 months and then commercial monkey chow for 18 months. The plasma cholesterol concentration in this group was 671 ± 19 mg/dl while the monkeys received the atherogenic diet and 127 ± 10 mg/dl when they received the normal diet. We have described this primate model of regression of atherosclerosis and the morphological changes that occur in the carotid and intracranial circulations in detail previously.3

Animals were sedated with 12 mg/kg i.v. ketamine and then anesthetized with 75–100 mg/kg i.v. α-chloralose. Supplemental anesthesia was administered as needed. The trachea was cannulated, and the monkey was ventilated mechanically with room air and supplemental oxygen.

A catheter was inserted into a femoral artery and advanced into the aorta for measurement of pressure and sampling of arterial blood. A femoral vein was cannulated for infusion of supplemental anesthesia. Two catheters were inserted into the left atrial appendage for injection of microspheres and infusion of serotonin. Catheters were also placed into both brachial arteries for withdrawal of reference blood samples during the injection of microspheres. Rectal temperature was monitored and maintained at 37–38°C with a heating pad. Most of the animals in these experiments were also used to examine vascular responses of the hind limb.13

Blood flow was measured four times in each monkey, using radioactive microspheres 15 μm in diameter as described in detail for monkeys.3,11 Briefly, microspheres were injected into the left atrium and reference arterial blood samples were withdrawn from the brachial arteries. At the end of the experiment, anesthetized animals were killed with intravenous KCl and the eyes were removed and prepared for gamma counting. The method for preparation of the retina for counting has been described in detail previously.9

Blood flow was measured under control conditions, during the infusion of 8 and 40 μg · kg⁻¹ · min⁻¹ serotonin into the left atrium, and following a 30-minute recovery period. Serotonin was infused into the left atrium instead of into a vein to avoid metabolism of the amine in the pulmonary circulation.

Statistical analysis was performed using a one-way analysis of variance; p < 0.05 was considered significant. All values are presented as mean ± SEM.

Results

Values for arterial pressure, arterial blood gases, and pH in the three groups of monkeys are shown in Table 1. These variables were similar in the three groups and were not altered significantly during the infusion of serotonin.

Under control conditions, blood flow to the retina was similar in the normal, atherosclerotic, and regression monkeys (Figure 1). Infusion of serotonin did not change blood flow to the retina in normal monkeys. In contrast, serotonin produced a marked reduction in blood flow to the retina in atherosclerotic monkeys (Figure 1). Blood flow to the retina was reduced by 74 ± 12% and 81 ± 9% during the infusion of 8 and 40 μg · kg⁻¹ · min⁻¹ serotonin, respectively. Following a 30-minute recovery period, blood flow to the retina returned to control levels in the atherosclerotic animals (Figure 1). In atherosclerotic monkeys that had received the normal diet for 18 months, serotonin had no effect on blood flow to the retina.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal monkeys (n=6)</th>
<th>Atherosclerotic monkeys (n=5)</th>
<th>Regression monkeys (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic pressure (mm Hg)</td>
<td>87 ± 5</td>
<td>85 ± 5</td>
<td>83 ± 5</td>
</tr>
<tr>
<td>Paco₂ (mm Hg)</td>
<td>36 ± 1</td>
<td>38 ± 1</td>
<td>35 ± 2</td>
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<tr>
<td>Paco₂ (mm Hg)</td>
<td>143 ± 15</td>
<td>110 ± 24</td>
<td>107 ± 9</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.38 ± 0.02</td>
<td>7.35 ± 0.01</td>
<td>7.42 ± 0.02</td>
</tr>
<tr>
<td>Control</td>
<td>87 ± 5</td>
<td>85 ± 5</td>
<td>83 ± 5</td>
</tr>
<tr>
<td>8</td>
<td>87 ± 7</td>
<td>90 ± 4</td>
<td>82 ± 4</td>
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<tr>
<td>40</td>
<td>85 ± 6</td>
<td>91 ± 5</td>
<td>81 ± 5</td>
</tr>
<tr>
<td>Recovery</td>
<td>80 ± 4</td>
<td>81 ± 1</td>
<td>83 ± 4</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

#### Table 1. Arterial Pressure, Blood Gases, and pH in Monkeys

**Sampling period**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>8</th>
<th>40</th>
<th>Recovery</th>
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<tbody>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>87 ± 5</td>
<td>87 ± 7</td>
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<tr>
<td>Paco₂ (mm Hg)</td>
<td>36 ± 1</td>
<td>36 ± 2</td>
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<tr>
<td>Paco₂ (mm Hg)</td>
<td>143 ± 15</td>
<td>128 ± 12</td>
<td>130 ± 12</td>
<td>128 ± 12</td>
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<tr>
<td>Arterial pH</td>
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<td>7.44 ± 0.02</td>
<td>7.38 ± 0.01</td>
<td>7.38 ± 0.01</td>
</tr>
</tbody>
</table>
Faraci et al
Regression of Atherosclerosis

Normal Blood Flow, (ml/min • 100g−1)

Atherosclerotic

Regression

FIGURE 1. Blood flow to retina under control conditions (C), during infusion of two doses of serotonin (μg • kg−1 • min−1), and following 30-minute recovery period (R) in normal, atherosclerotic, and regression monkeys. Values are mean ± SEM. *p<0.05 different from control value.

Blood flow to the retina was increased during the recovery period with respect to the initial control value in the regression group. Thus, we also compared values for blood flow during the infusion of serotonin with a combined control value (average of the control and recovery values). When analyzed in this manner, blood flow to the retina was reduced by 29±6% and 23±12%, from a control value of 139±25 ml • min−1 • 100 g−1, during the infusion of 8 and 40 μg • kg−1 • min−1 serotonin, respectively. Regardless of the manner in which the data are analyzed, these findings suggest that the augmented responses of retinal blood vessels to serotonin are largely restored to normal following the regression of atherosclerosis.

Discussion

The major findings of this study are that atherosclerosis greatly potentiates constrictor responses of the eye to serotonin and that dietary treatment of atherosclerosis reverses this abnormal response to serotonin.

Atherosclerosis potentiates vasoconstrictor responses of large arteries to products released by platelets (serotonin and thromboxane) and leukocytes (prostaglandin E2).3,4,11,14 There is substantial evidence for endothelial dysfunction in atherosclerotic arteries. Atherosclerosis impairs the basal and agonist-induced production or release of EDRF.7-8,15 Endothelium-dependent relaxation to aggregating platelets in large arteries is also impaired during hypercholesterolemia.16 Augmented responses to serotonin and impairment of dilator responses to endothelium-dependent agonists can occur in the microcirculation in the absence of intimal thickening.17-19 as well as in large arteries with atherosclerotic lesions.

In several blood vessels, serotonin has been shown to cause the release of EDRF.20 In cerebral arteries, removal of the endothelium augments contractile responses to serotonin,21 and hemoglobin, which binds EDRF, produces endothelium-dependent enhancement of contraction to serotonin in vitro.22 Removal of the endothelium also augments contraction in response to serotonin in the ophthalmic artery.23 Constrictor responses to serotonin are enhanced in the basilar artery from hypercholesterolemic rabbits, and this enhancement is reversed by administration of L-arginine, the precursor of EDRF.24 Thus, it seems likely that impairment of endothelium-dependent relaxation in atherosclerotic arteries contributes to augmented vasoconstriction in response to serotonin.

We have shown recently that atherosclerosis greatly augments constrictor responses of the retina to serotonin and thromboxane.49 Vasoconstrictor responses of the eye to activation of platelets in vivo are also potentiated during atherosclerosis.14 Vascular responses of the eye do not appear to be altered in a nonspecific manner, however, because responses to phenylephrine are not altered during atherosclerosis.9

Serotonin produces marked reductions in blood flow to the retina in atherosclerotic monkeys.6.9 This decrease in blood flow to the retina in atherosclerotic monkeys is associated with reversible impairment of the normal response of the retina to light (as measured by an electroretinogram).9 Thus, reductions in blood flow to the eye in response to serotonin are important functionally.

We have estimated that the high dose of serotonin used in this study produces a plasma concentration of 215 ng/ml.9 For comparison, occlusion of a coronary artery with a thrombus increases the level of serotonin in blood distal to the occlusion to more than 200 ng/ml.25 It is likely that the local concentration of serotonin at the site of platelet aggregation is even greater. These calculations suggest that the concentration of serotonin that was produced in this study is similar to that which may occur under pathophysiological conditions.

The present study demonstrates that regression of atherosclerosis abolishes augmented responses of the retinal circulation to serotonin. We have shown previously that dietary treatment of atherosclerosis restores the endothelium-dependent relaxation to normal and abolishes the augmented responses of large cerebral arteries and large arteries of the hind limb to serotonin.3,11,12

We and others have suggested that altered responses to products released by platelets during aggregation may contribute to enhanced vasoconstriction during
Thus, if constriction of retinal blood vessels in response to aggregating platelets plays a role in the pathophysiology of amaurosis fugax, dietary treatment of atherosclerosis may be successful in reducing the susceptibility to amaurosis. It is interesting to note that amaurosis fugax and transient ischemic attacks did not recur in patients with carotid atherosclerosis who received treatment to reduce their levels of plasma cholesterol.26

Acknowledgment
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References

KEY WORDS: cerebral blood flow · retina · serotonin · monkeys
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