Atherosclerosis Potentiates Constrictor Responses of Cerebral and Ocular Blood Vessels to Thromboxane in Monkeys

Frank M. Faraci, PhD, J. Koudy Williams, DVM, Keith R. Breese, Mark L. Armstrong, MD, and Donald D. Heistad, MD

The goal of our study was to examine the effects of infusion of serotonin and the thromboxane A₂ analogue U46619 into one carotid artery to simulate their release from platelets during aggregation. We measured blood flow to the brain and eye using microspheres and cerebral microvascular pressure in the pial arteries of normal and atherosclerotic cynomolgus monkeys. Unilateral intracarotid infusion of 10–30 μg/min serotonin did not affect cerebral blood flow in normal or atherosclerotic monkeys; serotonin did not alter blood flow to the eye in normal monkeys but decreased flow to the retina and choroid in atherosclerotic monkeys by 39±11% and 44±10% (mean±SEM), respectively. Infusion of 30 ng/min U46619 did not alter cerebral blood flow but increased the pressure gradient from the aorta to the pial artery, which is an index of large-artery resistance, in atherosclerotic monkeys. U46619 had no effect on blood flow to the eye in normal monkeys but decreased blood flow to the retina and choroid by 71±14% and 53±13%, respectively, in atherosclerotic monkeys. Thus, atherosclerosis potentiates the constrictor responses of large cerebral arteries to thromboxane and the responses of blood vessels of the eye to thromboxane and serotonin. (Stroke 1989;20:242–247)

Thromboxane is a potent constrictor of large cerebral arteries in vitro¹-⁴ and of pial vessels in vivo.⁵-⁷ Cerebral blood vessels may be exposed to thromboxane produced within the blood vessel wall or to thromboxane released by platelets during aggregation. ⁵-¹¹ Atherosclerotic blood vessels appear to produce approximately twice as much thromboxane as normal vessels.¹² Atherosclerosis potentiates cerebral vasoconstrictor responses to the platelet product serotonin.¹³,¹⁴ Impairment of endothelium-dependent relaxation⁹,¹⁵,¹⁶ may contribute to augmented vasoconstrictor responses to serotonin. In coronary and cerebral blood vessels, endothelium-derived relaxing factor (EDRF) may be released by thromboxane so that constrictor responses to thromboxane or an analogue are augmented after removal of the endothelium.¹⁷,¹⁸ Because endothelium-dependent relaxation is impaired by atherosclerosis, we tested the hypothesis that responses to thromboxane may be potentiated.

The goal of our study was to examine the effects of unilateral carotid infusion of the thromboxane A₂ analogue U46619 and serotonin to simulate their release from platelets during aggregation. One hypothesis was that atherosclerosis potentiates constrictor responses to thromboxane and serotonin; a secondary hypothesis was that constriction of one carotid artery is sufficient to affect cerebral and ocular blood flow.

Materials and Methods

Animal Preparation

We used two groups of adult cynomolgus monkeys. The eight normal monkeys were fed commercial chow (Purina monkey chow, Ralston Purina, Richmond, Indiana), which produces plasma cholesterol concentrations of 100–130 mg/dl.¹³-¹⁵ In nine monkeys, atherosclerosis was induced by feeding them an atherogenic diet that contained 41% of total calories from fat and 0.8% cholesterol for 18 months; mean±SEM plasma cholesterol concentrations were 645±34 mg/dl during the last 3 months.

Each monkey was sedated with 12 mg/kg ketamine and then anesthetized with 75–100 mg/kg i.v.
chioralose. Supplemental anesthesia was administered as needed. The trachea was cannulated, and the monkey was ventilated mechanically with room air and supplemental oxygen. In normal monkeys, mean±SEM PaCO₂ was 38±1 mm Hg, PaO₂ was 110±6 mm Hg, and arterial pH was 7.46±0.02. In atherosclerotic monkeys, mean±SEM PaCO₂ was 37±1 mm Hg, PaO₂ was 110±3 mm Hg, and arterial pH was 7.45±0.01. These values did not change significantly during the experiments.

A catheter was inserted into a femoral artery and advanced into the descending thoracic aorta for measurement of aortic pressure and to sample arterial blood. A femoral vein was cannulated for infusion of serotonin and U46619. Catheters were inserted into the left atrial appendage for injection of microspheres and into both brachial arteries for withdrawal of reference blood samples during microsphere injection. The skeletal muscles were paralyzed with 10 mg/kg gallamine triethiodide. Rectal temperature was monitored and maintained at 37–38° C with a heating pad.

The right external carotid artery was exposed and ligated at the carotid bifurcation. A catheter was inserted into the lingual artery for infusion of serotonin and U46619 at a rate of 0.2 ml/min into the carotid artery. A ligature was placed loosely around the right common carotid artery and was tightened for 2 minutes to produce carotid occlusion.

Blood flow was measured using radioactive microspheres 15 μm in diameter as described in detail for monkeys. The at the end of the experiment, anesthetized monkeys were killed with intravenous KCl. Catheters were inserted into the left atrial appendage for injection of microspheres and into both brachial arteries for withdrawal of reference blood samples during microsphere injection. The skeletal muscles were paralyzed with 10 mg/kg gallamine triethiodide. Rectal temperature was monitored and maintained at 37–38° C with a heating pad.

After insertion of the catheters, each monkey was placed in a head holder. A craniotomy was made to expose the vessels over the right parietal cortex as described in detail. The dura was incised to expose the pial vessels over the cerebrum. Artificial cerebrospinal fluid (CSF), equilibrated with gas mixtures and warmed to 37° C, was continually suffused over the exposed portion of the brain. For both groups of monkeys combined, CSF sampled from the craniotomy had a mean±SEM PCO₂ of 36±1 mm Hg, a PO₂ of 41±3 mm Hg, and a pH of 7.42±0.01.

Pial artery pressure was measured using sharpened micropipettes with 2–4-μm tip diameters filled with 1.5 M NaCl and coupled to a servo-null pressure measuring device (Model 4A, Instrumentation for Physiology and Medicine, Inc., San Diego, California). The tip of the micropipette was inserted into the lumen of a pial artery on the cerebrum using a micromanipulator. Vessels were observed using a microscope coupled to a video camera and video recorder. Diameter of the pial arteries was measured with an electronic micrometer. Pressure was measured in pial arteries with a mean±SEM diameter of 363±36 μm in the normal monkeys and of 333±33 μm in the atherosclerotic monkeys.

**Experimental Protocol**

Pial artery pressure, cerebral blood flow, and ocular blood flow were measured under control conditions, during unilateral infusion of serotonin (Sigma Chemical Co., St. Louis, Missouri), during unilateral carotid artery occlusion, and during unilateral infusion of U46619 (15S-hydroxy-11α,9α-epoxymethanoprosta-SZn-dienoic acid; The Upjohn Co., Kalamazoo, Michigan).

In three normal and three atherosclerotic monkeys, we infused 3 and 10 μg/min serotonin. In five normal and six atherosclerotic monkeys, we infused 10 and 30 μg/min serotonin. In both sets of animals, pial artery pressure was measured during infusion of both doses, but blood flow was measured only during infusion of the high dose. In eight normal and eight atherosclerotic monkeys, 10 and 30 ng/min U46619 was infused after recovery from unilateral carotid artery occlusion. The volume infused was 0.2 ml/min.

Measurements were made as follows. First, blood flow and pial artery pressure were measured under control conditions. Second, pial artery pressure was measured during infusion of the low dose of serotonin but blood flow was not measured; flow and pressure were measured during infusion of the high dose of serotonin. Third, blood flow and pial artery pressure were again measured under control conditions. Fourth, blood flow and pial artery pressure were measured during unilateral carotid artery occlusion. Pial artery pressure returned to control levels following occlusion. Fifth, pial artery pressure was measured during infusion of 10 ng/min U46619, and both pial artery pressure and blood flow were measured during infusion of 30 ng/min U46619.

In blood samples obtained from four normal and four atherosclerotic monkeys, we determined the concentration of U46619 that was sufficient to aggregate platelets in vitro using a platelet aggregometer, which detects an increase in transmitted light during second-order platelet aggregation.

Statistical analysis was performed using paired t tests, as values during interventions were compared with the preceding control value. Bonferroni's correction was used for multiple comparisons. A probability value of <0.05 was considered significant. All values are presented as mean±SEM.

**Results**

In normal monkeys, the common and internal carotid arteries were thin-walled, with no gross or microscopic evidence of atherosclerotic lesions. In
TABLE 1. Effect of Serotonin, Carotid Artery Occlusion, and U46619 on Blood Flow to Brain and Eye in Monkeys

<table>
<thead>
<tr>
<th>Blood flow (ml/min/100 g)</th>
<th>Control</th>
<th>Serotonin</th>
<th>Control</th>
<th>Occlusion</th>
<th>U46619</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrum</td>
<td>39±3</td>
<td>37±3</td>
<td>40±3</td>
<td>43±4</td>
<td>44±6</td>
</tr>
<tr>
<td>Retina</td>
<td>83±14</td>
<td>92±24</td>
<td>96±22</td>
<td>80±20</td>
<td>73±9</td>
</tr>
<tr>
<td>Choroid</td>
<td>2351±595</td>
<td>2128±477</td>
<td>2866±767</td>
<td>2759±866</td>
<td>2930±999</td>
</tr>
<tr>
<td>Atherosclerotic (n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrum</td>
<td>34±3</td>
<td>33±3</td>
<td>36±3</td>
<td>37±4</td>
<td>39±4</td>
</tr>
<tr>
<td>Retina</td>
<td>71±14</td>
<td>37±7*</td>
<td>57±8</td>
<td>59±17</td>
<td>26±11*</td>
</tr>
<tr>
<td>Choroid</td>
<td>3311±648</td>
<td>1745±467*</td>
<td>2738±541</td>
<td>2531±695</td>
<td>1257±477*</td>
</tr>
</tbody>
</table>

Values are mean±SEM.  
*Significantly different from preceding control at p<0.05.

Atherosclerotic monkeys, lesions of the common and proximal internal carotid arteries were observed that ranged from fatty streaks to fibrofatty plaques. Microscopic examination showed diffuse lesions throughout the common and proximal internal carotid arteries. Microscopic examination of serial sections of the ophthalmic artery in five atherosclerotic monkeys revealed the presence of atherosclerotic lesions in three. Atherosclerotic lesions were not observed in the ophthalmic arteries from four normal monkeys.  

Unilateral intracarotid infusion of 10–30 μg/min serotonin had no effect on cerebral blood flow in normal or atherosclerotic monkeys (Table 1). Serotonin did not alter blood flow to the retina and choroid of the eye in the normal monkeys, but it did decrease retinal and choroidal blood flow significantly in the atherosclerotic monkeys (Figure 1). Unilateral intracarotid infusion of U46619 also had no effect on cerebral blood flow in normal or atherosclerotic monkeys (Table 1). U46619 had no effect on blood flow to the eye in the normal monkeys, but it did produce a marked reduction in blood flow to the retina and choroid in the atherosclerotic monkeys (Figure 2). Unilateral carotid artery occlusion had no effect on blood flow to the brain or eye in normal or atherosclerotic monkeys (Table 1).  

The pressure gradients between the aorta and the pial arteries were similar in both groups of monkeys under control conditions (Table 2). Unilateral intracarotid infusion of serotonin had no significant effect on microvascular pressure in normal or atherosclerotic monkeys (Table 2). Unilateral intracarotid infusion of U46619 did not alter pial artery pressure in the normal monkeys but produced a significant decrease in pial artery pressure in the atherosclerotic monkeys (Figure 3, Table 2). Infusion of 30 ng/min U46619 increased the pressure gradient by 6.6±2.8 mm Hg relative to control. Unilateral carotid artery occlusion increased the pressure gradient by 8.0±0.9 mm Hg in the normal monkeys and by 9.6±2.4 mm Hg in the atherosclerotic monkeys. The response to infusion of 30 ng/min U46619 in atherosclerotic monkeys was 66±19% of that produced by carotid artery occlusion. U46619 produced second-order aggregation of platelets in blood from both groups of monkeys. This effect was produced at similar concentrations of U46619 in blood from normal and atherosclerotic monkeys (4.3±0.5×10⁻⁶ M and 4.8±1.3×10⁻⁶ M, respectively).
TABLE 2. Effect of Serotonin, Carotid Artery Occlusion, and U46619 on Cerebral Microvascular Pressure in Monkeys

<table>
<thead>
<tr>
<th>Pressure (mm Hg)</th>
<th>Serotonin (μg/min)</th>
<th>U46619 (ng/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 10 30</td>
<td>Control Occlusion 10 30</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>88±6</td>
<td>89±6</td>
</tr>
<tr>
<td>Pial artery</td>
<td>67±4</td>
<td>67±4</td>
</tr>
<tr>
<td>Gradient</td>
<td>22±4</td>
<td>22±3</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Atherosclerotic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>82±3</td>
<td>82±3</td>
</tr>
<tr>
<td>Pial artery</td>
<td>58±3</td>
<td>57±4</td>
</tr>
<tr>
<td>Gradient</td>
<td>24±2</td>
<td>25±2</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n, number of monkeys.

*Significantly different from preceding control at p<0.05.

Discussion

Our major finding was that intracarotid infusion of the thromboxane A2 analogue U46619 decreased pial artery pressure and produced a marked decrease in blood flow to the retina and choroid in atherosclerotic but not in normal monkeys. A secondary finding was that infusion of serotonin and thromboxane into only one carotid artery was sufficient to affect blood flow to the eye.

In our previous studies, systemic or bilateral carotid infusion of serotonin decreased cerebral microvascular pressure, especially in atherosclerotic monkeys. In contrast, in this study pial artery pressure was not affected by unilateral carotid infusion of serotonin in normal or atherosclerotic monkeys. It is likely that, when serotonin is infused into only one carotid artery, compensatory responses of the other major inflow vessels are adequate to maintain microvascular pressure at control levels. These compensatory responses are not adequate to prevent decreases in pial artery pressure when more than one vessel is constricted, as occurs during bilateral carotid infusion or systemic administration of serotonin.

In our present study, infusion of U46619 into one carotid artery in normal monkeys had no effect on pial artery pressure or cerebral blood flow and had no effect on blood flow to the eye. A major new finding of this study is that constrictor responses of large cerebral arteries and blood vessels of the eye to thromboxane are potentiated by atherosclerosis. In the atherosclerotic monkeys, U46619 was a very potent vasoconstrictor since the reduction in pial arterial pressure was two thirds that produced by unilateral carotid artery occlusion.

U46619 produced aggregation of platelets at similar concentrations in blood from both normal and atherosclerotic monkeys. These findings suggest that the difference in responses to U46619 in normal and atherosclerotic monkeys was not due to differences in platelet aggregation and thus was the result of differences in vascular responses.

Several mechanisms may contribute to the augmented constrictor responses to serotonin and U46619 in atherosclerotic monkeys. First, changes in the membrane cholesterol of vascular muscle may increase vascular responses. Responses do not appear to be augmented in a nonspecific way, however.

Atherosclerosis may alter the number or sensitivity of receptors for serotonin and thromboxane. Thus, it is possible that changes in the receptor function in blood vessels of the brain and eye contribute to the potentiation of responses by atherosclerosis.

Atherosclerosis impairs endothelium-dependent relaxation to several stimuli. Constrictor responses to serotonin are augmented following removal of the endothelium from normal arteries. Although one report suggests that removal of the endothelium does not alter constriction to U46619, several other reports suggest that constrictor responses to thromboxane analogues are potentiated by removal of the endothelium. These data...
are compatible with the hypothesis that thrombox-
ane releases EDRF. Because atherosclerosis impairs
endothelium-dependent relaxation, it is likely that
augmented vasoconstrictor responses to thrombox-
ane are related in part to this mechanism.

The precise site of vasoconstriction in response
to serotonin and thromboxane is not known. Pre-
sumably, blood vessels such as the carotid arteries
and the ophthalmic artery, which had atheroscle-
rotic lesions, were the sites of increased vasocon-
striction. We cannot exclude the possibility, how-
ever, that smaller distal blood vessels, such as those
in the retina or choroid, exhibited abnormal
responses as well. Although the precise site of
vasoconstriction has not been defined, our major
finding is that both serotonin and a thromboxane
analogue produced marked decreases in blood flow
to the eye during atherosclerosis.

During adherence and aggregation at atheroscle-
rotic lesions, activated platelets release vasoactive
products, including serotonin and thromboxane.8,9
Release of serotonin and thromboxane during platelet
aggregation in large extracranial arteries may con-
strict large arteries and reduce pial artery pressure.
We speculate that, in the presence of a stenosis or
partial obstruction of a cerebral artery by an embolus,
a reduction in microvascular pressure may contribute
to cerebral ischemia during transient ischemic attacks.
Our findings suggest that thromboxane may play a
more important role than serotonin in the pathogene-
sis of cerebral ischemia.

We have suggested that the release of serotonin
from activated platelets may contribute to amauro-
sis fugax during atherosclerosis.19 Serotonin-
induced decreases in retinal blood flow were asso-
ciated with marked decreases in the amplitude of the
electroretinogram. Our results suggest that
thromboxane as well as serotonin may produce
exaggerated vasoconstriction in the ocular circula-
tion, resulting in marked reductions in retinal blood
flow, which could contribute to amaurosis fugax.

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