Cerebral Vasoconstrictor Responses to Serotonin
After Dietary Treatment of Atherosclerosis:
Implications for Transient Ischemic Attacks

Donald D. Heistad, MD, Keith Breese, and Mark L. Armstrong, MD

Serotonin, which is released when platelets aggregate at carotid lesions, may contribute to cerebral ischemia. Our goal was to test the hypothesis that dietary treatment of atherosclerosis reverses the augmented cerebral vasoconstrictor response to serotonin. We studied normal cynomolgus monkeys, atherosclerotic monkeys, and atherosclerotic monkeys that were fed a normal (regression) diet for 18 months. Morphometric studies indicated that the regression diet reduced intimal area in the carotid arteries by about 50-75%. Cerebral blood flow was measured with microspheres, and microvascular pressure was measured with a micropipette in pial arteries that were approximately 300 μm in diameter. Values for cerebral blood flow and arteriolar pressure were used to calculate resistance of large cerebral arteries (>300 μm diameter). Infusion of serotonin produced a modest increase in the resistance of large cerebral arteries in normal monkeys. Vasoconstrictor responses to serotonin were increased more than fivefold in atherosclerotic monkeys. The major finding of the study is that dietary treatment of atherosclerosis abolishes augmented cerebral responses to serotonin. (Stroke 1987; 18:1068-1073)

Large arteries as well as arterioles play an important role in the regulation of cerebral blood flow (CBF). Hypercholesterolemia in non-human primates produces atherosclerotic lesions in large extracranial arteries with relative sparing of intracranial arteries including extraparenchymal vessels. We have observed that diet-induced atherosclerosis in primates impairs cerebral vasodilator responses and potentiates vasoconstrictor responses to serotonin.

Transient ischemic attacks (TIAs) usually are produced by platelet adhesion, aggregation, and embolization from plaques in large extracranial arteries. Platelets contain large amounts of serotonin. Release of serotonin during the aggregation of platelets, coupled with augmented responses to serotonin in atherosclerotic arteries, may produce pronounced vasoconstriction and contribute to focal cerebral ischemia.

Dietary treatment of atherosclerosis in primates results in reduction of lesion size. Although the intima remains thickened compared with normal vessels, cerebral vasodilator responses to hypercapnia are improved significantly. It is not known whether dietary treatment of atherosclerosis reduces the hyperresponsiveness of cerebral vessels to constrictor stimuli.

The goal of this study was to test the hypothesis that dietary treatment of atherosclerosis in primates reduces the hyperresponsiveness of cerebral vessels to serotonin.

Materials and Methods

Three groups of male cynomolgus monkeys were studied. Seven normal monkeys were fed commercial laboratory chow (Purina Monkey Chow, Ralston Purina Co., Richmond, Ind.) (normal group). Eight monkeys were fed an atherogenic diet, which contained 41% of total calories as fat and 0.8% cholesterol, for 18-20 months (atherosclerotic group). Eight monkeys were fed the same atherogenic diet for 18 months and then were fed commercial laboratory chow for 18-20 months (regression group). The monkeys weighed 5.9±0.2 kg (mean±SEM). At intervals of 3-4 months, the monkeys were sedated with 10 mg/kg i.m. ketamine HCl and venous blood samples were obtained. Total cholesterol and triglycerides were determined with the protocol used by the Lipid Research Clinics for the Autoanalyzer II (Technicon Instruments, Inc., Tarrytown, N.Y.).

At the time of study, the monkeys were sedated with 12 mg/kg i.m. ketamine and anesthetized with 75-100 mg/kg i.v. chloralose. The monkeys were intubated and ventilated with room air and supplemental oxygen. When variability of arterial pressure or heart rate increased, supplemental doses of chloralose were injected. The monkeys usually required 50 mg/kg i.v. after completion of surgery (about 4 hours after induction of anesthesia) and 50 mg/kg 1-2 hours later. Rectal temperature was maintained at 37-38°C with a heating pad.

A catheter was inserted through a femoral artery to the descending aorta to measure aortic pressure and to obtain blood samples. Catheters were inserted into
Measurement of Pial Artery Pressure and Cerebral Blood Flow

Catheters also were inserted into the femoral and axillary veins for injection of fluids and drugs. Through a thoracotomy, 2 catheters were inserted into the left atrium; one was used for injection of microspheres and the other for infusion of serotonin. A right thoracotomy also was performed to minimize changes in cerebral volume during ventilation. A ligature was placed loosely around the descending aorta, below the tip of the aortic catheter.

Heparin (500 U/kg) was given i.v. after completion of the surgical procedures.

Experimental Protocol

Pial artery pressure and CBF were measured during a control period, during infusion of serotonin into the left atrium, during a second control period, and during systemic hypcapnia. We infused 8 and 40 μg/kg/min serotonin into the left atrium. Hypcapnia was produced by increasing the rate and tidal volume of the respirator. Serotonin and hypcapnia tended to reduce arterial pressure in several normal and regression monkeys, and a ligature around the descending aorta was tightened to keep arterial pressure in the upper half of the body from falling. Serotonin tended to increase arterial pressure in several atherosclerotic monkeys, and blood was removed during infusion of serotonin in these monkeys to prevent this increase in arterial pressure.

Serotonin was infused for about 3 minutes before microspheres were injected, and the infusions were continued for about 10 minutes after injection of microspheres. Hypcapnia was maintained for about 15 minutes before microspheres were injected. We waited for at least 15 minutes between interventions.

In normal monkeys, Paco2 was 39 ± 0.5 mm Hg, Po2 was 106 ± 7 mm Hg, and pH was 7.41 ± 0.01 during control, and, except during hypcapnia, these values did not change significantly. During hypcapnia, Paco2 decreased to 16 ± 0.9 mm Hg and pH increased to 7.67 ± 0.05. In atherosclerotic monkeys, Paco2 was 38 ± 0.2 mm Hg, Po2 was 105 ± 3 mm Hg, and pH was 7.43 ± 0.01 during control conditions; Paco2 decreased to 18 ± 0.6 mm Hg and pH increased to 7.61 ± 0.03 during hypcapnia. In regression monkeys, Paco2 was 38 ± 0.6 mm Hg, Po2 was 106 ± 7 mm Hg, and pH was 7.44 ± 0.02; Paco2 decreased to 18 ± 0.7 mm Hg and pH increased to 7.63 ± 0.02 during hypcapnia. Control values for artificial CSF in all 3 groups were maintained at levels close to Paco2 = 40 mm Hg, Po2 = 40–50 mm Hg, and pH = 7.35. During hypcapnia, Paco2 of artificial CSF was approximately 16 mm Hg and pH approximately 7.60.

CBF was calculated as CBF = [(counts/g brain × 100 × withdrawal rate of reference blood samples)/counts in reference blood samples]. Large artery resistance (LAR) was calculated as LAR = [(aortic pressure− pial artery pressure)/CBF].

Morphologic Studies

The carotid arteries, vertebral arteries, and circle of Willis were removed, examined for gross lesions, and fixed in buffered formalin. Histologic study of the carotid arteries was carried out on paraffin sections of preselected sites. Sections were stained with hematoxylin and eosin and Verhoeff-Van Gieson stain. Morphometric determination of the size of the intima and media was performed with an image analyzer as described previously.

Statistical Analysis

Values for interventions were compared with the preceding control period with a one-way analysis of variance. Mean intergroup differences between normal, atherosclerotic, and regression monkeys were tested for significance with the Student-Newman-Keuls procedure.
Table 1. Intimal Area in Normal, Atherosclerotic, and Regression Monkeys

<table>
<thead>
<tr>
<th>Carotid artery</th>
<th>Normal</th>
<th>Atherosclerotic</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>0.06±0.06</td>
<td>1.92±0.48*</td>
<td>0.88±0.22</td>
</tr>
<tr>
<td>Middle</td>
<td>0</td>
<td>1.88±0.69*</td>
<td>0.43±0.12</td>
</tr>
<tr>
<td>Distal</td>
<td>0</td>
<td>1.76±0.50*</td>
<td>0.58±0.10</td>
</tr>
<tr>
<td>Vertebral artery</td>
<td>0.87±0.20*</td>
<td>0.28±0.10</td>
<td></td>
</tr>
</tbody>
</table>

Values are μm², mean±SEM. *p<0.05 vs. normal and regression monkeys.

Results

Plasma cholesterol was 96 ± 5 mg/dl in normal monkeys and 531 ± 39 mg/dl during the last 3 months of atherogenic diet in atherosclerotic monkeys. Plasma cholesterol in regression monkeys was 539 ± 47 mg/dl while they received atherogenic diet and 118 ± 10 when they received normal diet. Plasma triglycerides were <40 mg/dl in normal, atherosclerotic, and regression monkeys.

Morphologic Changes

In atherosclerotic monkeys, morphologic changes were similar to those described previously. The common and internal carotid arteries had pronounced atherosclerotic changes, with diffuse thickening of the vessel wall by virtually continuous lesions. The common carotid arteries demonstrated fibrofatty lesions with necrosis and calcification. Fatty streaks were the dominant lesion type in the extracranial internal carotid arteries. Intracranial arteries usually were virtually normal; lesions, when present, were limited to small fatty streaks. In regression monkeys, lipids were virtually absent in the extracranial arteries, but substantial fibrosis was present.

Morphometric study demonstrated significant increases in intimal area of the common carotid and vertebral arteries of atherosclerotic monkeys, with no significant increase in the intimal area of regression monkeys (Table 1). In both the carotid and vertebral arteries, medial area was similar in normal, atherosclerotic, and regression monkeys.

Cerebral Hemodynamics During Control Conditions

Before the interventions, pressure in pial arteries approximately 300 μm in diameter was 18–25 mm Hg lower than aortic pressure in normal, atherosclerotic, and regression monkeys (Table 2). Thus, large arteries (>300 μm in diameter) account for 25–30% of the vascular resistance in the cerebral circulation. This finding provides further support for the concept that large arteries as well as arterioles contribute importantly to cerebral vascular resistance in primates and other species.

Values for CBF obtained during the first control period appeared to be lower in regression monkeys than in normal or atherosclerotic monkeys (Table 2). This finding probably is of little importance because CBF during the second control period was similar in the 3 groups (Table 2).

Responses to Serotonin

The difference between aortic and pial arterial pressure increased during infusion of serotonin (Table 2, Figure 1). Serotonin reduced pial artery pressure (Fig-
Heistad et al  Treatment of Atherosclerosis

Normal Atherosclerotic Regression

A Pressure

Aorta to Pial Artery

10

8 40 8 40 8 40

8 40 8 40 8 40

μg Serotonin/kg per min

**FIGURE 1.** Cerebral vascular responses to infusion of serotonin into left atrium in normal, atherosclerotic, and regression monkeys. Values (mean±SEM) indicate effects of serotonin on drop in pressure from aorta to pial arteries approximately 300 μm in diameter. *p<0.05 vs. normal and regression monkeys.

This effect was small in normal monkeys and much larger in atherosclerotic monkeys. The decrease in pressure from aorta to pial artery increased more during infusion of 8 μg/kg/min in atherosclerotic monkeys than during infusion of 40 μg/kg/min in normal monkeys (Figure 1). Thus, in terms of relative potency, responses to serotonin were augmented more than fivefold in atherosclerotic monkeys. Responses to serotonin were restored to normal (Figures 1 and 2) after dietary treatment of atherosclerosis.

LAR in mm Hg/ml·min·100 mg was 0.6 ±0.1 in normal monkeys, 1.0 ±0.3 in atherosclerotic monkeys, and 1.2 ±0.1 in regression monkeys under control conditions. Infusion of serotonin increased LAR; there was a modest increase in normal monkeys and a much greater increase in atherosclerotic monkeys. Increases in LAR during infusion of serotonin were restored to virtually normal after regression of atherosclerosis (Figure 3).

Thus, in normal monkeys, as described previously in other animals, serotonin constricted large arteries and reduced cerebral microvascular pressure. Atherosclerosis potentiated constrictor responses of large cerebral arteries, and dietary treatment of atherosclerosis abolished the hyperresponsiveness to serotonin.

**Responses to Hypocapnia**

To test the specificity of altered cerebral vascular responses, effects of hypocapnia were examined. Hyperventilation significantly reduced CBF in normal, atherosclerotic, and regression monkeys (Table 2, Figure 4); the reduction was similar in the 3 groups. Hypocapnia did not alter the decrease in pressure from the aorta to pial artery in normal, atherosclerotic, and regression monkeys (Figure 4).

Thus, cerebral vasoconstrictor responses to hypocapnia were not altered by atherosclerosis or dietary treatment of atherosclerosis.

**Discussion**

We have suggested that the release of serotonin during aggregation of platelets at carotid lesions may produce vasoconstriction and contribute to the pathogenesis of transient cerebral ischemia. The major new finding in this study is that cerebral vasoconstrictor responses to serotonin, which were potentiated more than fivefold by atherosclerosis, were restored to normal by dietary treatment of atherosclerosis.
Methods

Insertion of a micropipette into pial arteries had no detectable effect on most vessels. Diameter of the vessel occasionally changed after insertion of the pipette, but changes were always transient. Hemorrhage at the puncture site occurred only when the pipette tip was removed from the vessel. No monkeys were excluded from the study because of spasm or hemorrhage produced by the pipette.

We measured blood flow to the entire ipsilateral cerebral hemisphere and measured pressure in a single pial artery. This approach (using a global measurement of blood flow and a local measurement of pressure) assumes that the craniotomy and micropipette do not alter blood flow to the region examined. In previous studies, we examined this assumption by separately measuring blood flow to brain tissue perfused by the pial artery in which we had measured pressure and in the remainder of the brain. Blood flow in these regions and the remainder of the cerebrum were similar under control conditions and during interventions. Thus, blood flow to the region under the pial window and to the area supplied by the punctured pial artery is similar to that to the entire hemisphere.

Serotonin constricts large extracranial arteries and thus decreases cerebral microvascular pressure. Despite constriction of large arteries, however, serotonin does not reduce CBF because small cerebral vessels dilate. The response of small vessels presumably is an autoregulatory response to the decrease in pial artery pressure. Thus, our experimental approach allowed detection of augmented vasoconstrictor responses to serotonin in atherosclerotic monkeys and restoration to normal during dietary treatment that would not have been detected if CBF, but not cerebral microvascular pressure, were measured.

The precise site of constriction of large arteries during infusion of serotonin is not known. Serotonin may constrict large extracranial arteries, or it may constrict large intracranial arteries proximal to the pial arteries in which pressure was measured. It seems likely that atherosclerotic alteration of responses to serotonin in this study occurred primarily in extracranial arteries because atherosclerotic lesions were confined to these vessels. Lipoproteins and hypercholesterolemia have been reported to alter the responses of pial arteries, however, even in the absence of atherosclerotic lesions. Thus, we cannot exclude the possibility that responses to serotonin are altered in large intracranial arteries, as well as in extracranial arteries, of atherosclerotic monkeys.

CBF was relatively low in this study, as in our previous studies of primates. The low values for CBF are not produced by arteriovenous shunting of microspheres in cats or dogs or in primates (F.M. Faraci and D.D. Heistad, unpublished observations). It is likely that the values for CBF were low because the monkeys were anesthetized and because the length of the experiment was usually 6–8 hours. These levels of blood flow probably do not compromise the validity of the findings, however, because vasoconstrictor responses, which were the focus of the study, were preserved.

Vascular Effects of Atherosclerosis and Regression

Primates develop atherosclerotic lesions in the carotid arteries that resemble those that occur in humans. There are marked increases in intimal area, with encroachment of the lesions into the lumen and impairment of maximal vasodilator responses. Regression of plaques in the carotid arteries has been demonstrated in humans. During dietary treatment of atherosclerosis in primates, intimal area is reduced as the lesions regress. There is a tendency for intimal fibrosis to progress, however, during dietary treatment of atherosclerosis so that in some vascular beds maximal vasodilator responses fail to improve.

In cerebral vessels, regression of atherosclerosis is accompanied by marked improvement in vasodilator responses.

Vasospasm clearly is an important complication of coronary atherosclerosis, but it is not clear whether atherosclerosis predisposes to vasospasm in other vascular beds, including the cerebral circulation. Changes in endothelial function may contribute to alteration of vascular responses and susceptibility to vasospasm in atherosclerotic arteries. Endothelium-dependent relaxing factor modulates responses to many vasoactive substances including exogenous serotonin and serotonin that is released by platelets. Recent studies indicate that atherosclerosis impairs endothelium-dependent vascular responses. Synthesis of endothelium-dependent relaxing factor is impaired in atherosclerotic vessels.

A preliminary report suggests that treatment of atherosclerosis restores endothelium-dependent vascular responses to normal. We speculate, therefore, that one mechanism by which hyperresponsiveness to serotonin in the cerebral circulation is abolished during dietary treatment of atherosclerosis may involve restoration of normal endothelium-dependent responses.

Vasoconstrictor responses to hypoxia in contrast to responses to serotonin, were not altered by atherosclerosis or dietary treatment. Thus, alteration of responses may be somewhat specific for serotonin.

We have estimated the concentration of serotonin that was achieved during its infusion in these experiments and compared this value with blood levels that have been observed in vivo. We assumed that 90% of the serotonin is cleared in 1 passage through the pulmonary circulation, that circulation time in monkeys is 14 seconds, and that the space of distribution is limited to plasma. We thereby estimated that infusion of 8 and 40 μg/kg/min serotonin into the left atrium produces plasma concentrations of 43 and 215 ng/ml. For comparison, partial occlusion of a coronary artery with a thrombus increases the level of serotonin in blood distal to the occlusion from 9 to 213 ng/ml.

Thus, the blood levels of serotonin that were achieved in this study probably are similar to those that may occur in pathophysiologic states. Furthermore, the concentration of serotonin in arterial segments under a
thrombus has been reported to increase to > 800 ng/g of vessel.13

Implications

Most TIAs apparently are produced by the aggregation of platelets in extracranial arteries.1 Release of serotonin during platelet aggregation may contribute to the pathogenesis of TIAs.6 Serotonin constricts large extracranial arteries and reduces distal pressure so that, in the presence of fixed stenosis of a small artery or partial obstruction by an embolus, it may contribute to focal cerebral ischemia. We now suggest that dietary treatment of atherosclerosis, by abolishing the hyper-responsiveness to serotonin in the cerebral circulation, may reduce susceptibility to TIAs.

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References


Key Words • atherosclerosis • serotonin • microvascular pressure • cerebral vessels

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