Effects of Atherosclerosis on Cerebral Vessels:
Hemodynamic and Morphometric Studies

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SUMMARY In this study hemodynamic and morphometric consequences of atherosclerosis were examined in cynomolgus monkeys. We tested the hypothesis that atherosclerosis augments cerebral vasoconstrictor responses to serotonin. We studied 8 normal and 8 atherosclerotic monkeys, which were fed an atherogenic diet for 17 months. Morphometric studies indicated marked intimal proliferation of extracranial carotid arteries, with only modest reduction in the vascular lumen, as atherosclerotic lesions were displaced outward. Cerebral blood flow was measured with microspheres and microvascular pressure was measured with a micropipette in pial arteries approximately 350 μm diameter. Intracarotid infusion of serotonin reduced microvascular pressure, which indicates constriction of large arteries upstream, but cerebral blood flow did not decrease. Serotonin produced a 2-fold greater reduction in cerebral microvascular pressure in atherosclerotic monkeys than in normal monkeys. Intracarotid histamine increased flow and hypopapnia reduced flow in both normal and atherosclerotic monkeys, without altering cerebral microvascular pressure. We conclude: First, atherosclerosis potentiates constrictor responses to serotonin in large cerebral arteries. Because platelets release serotonin when they aggregate, augmentation of responses by atherosclerosis may have implications for cerebral vascular responses during aggregation of platelets at carotid lesions. Second, despite marked proliferation of intima, atherosclerotic lesions are displaced outward during a prestenotic phase of the disease, so that the lumen is relatively well preserved.

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Atherosclerotic lesions occur initially in the aorta and large arteries, and then in progressively more distal arteries. Thus, in normotensive primates, the aorta and large arteries develop atherosclerotic lesions and intracranial arteries are relatively spared. Large arteries, as well as arterioles, play an important role in regulation of cerebral blood flow under physiological conditions and in atherosclerotic primates.

In other vascular beds, atherosclerotic lesions predispose to vasospasm. Several vasoactive substances have been proposed to be important in the pathogenesis of vasospasm, but the relative importance of these possible mediators is not clear. Studies in vitro indicate that atherosclerosis augments vasoconstrictor responses to serotoninergic stimuli and histamine. Studies in vivo also indicate that atherosclerosis potentiates vasoconstrictor responses to serotonin and histamine, and thus suggest that these amines may play a role in the pathogenesis of vasospasm.

Transient ischemic attacks (TIAs) usually are produced by aggregation of platelets in large extracranial arteries, with subsequent emboli or microemboli. Vasospasm does not appear to play an important role in the pathogenesis of TIAs. Nevertheless, platelets contain substantial concentrations of serotonin, and it is possible that release of serotonin during aggregation of platelets may contribute to cerebral vasospasm. We will suggest that constriction of large arteries by serotonin reduces cerebral microvascular pressure and, in the presence of subcritical distal stenosis, may thereby contribute to focal cerebral ischemia.

In this study we examined effects of atherosclerosis on cerebral vascular responses. Our hypothesis, based on previous studies of the limb and coronary circulation, was that atherosclerosis may potentiate cerebral vasoconstrictor responses to serotonin and histamine. Several aspects of this study are distinctive. First, we infused serotonin and histamine into the carotid arteries, to examine vascular responses and to simulate local release of these amines. Second, a primary model of atherosclerosis was studied. Third, in addition to measuring blood flow, we measured cerebral microvascular pressure downstream from large arteries. Measurement of microvascular pressure may be more sensitive than measurement of blood flow in detection of constrictor responses of large cerebral arteries. Fourth, we performed morphometric studies of the carotid arteries. Our goal was to determine the extent that atherosclerotic lesions encroach on the arterial lumen, and to determine whether outward displacement of the lesions helps to preserve the vascular lumen.

Methods

Two groups of adult cynomolgus monkeys were studied. Eight normal monkeys that weighed 6.9 ± 0.4 kg (mean ± SE) were fed commercial laboratory chow (Purina monkey chow, Ralston Purina Co.). In eight monkeys (weight = 5.7 ± 0.2 kg), atherosclerosis was induced by feeding them a semipurified atherogenic diet for 17 months. The diet contained 41% of total calories from fat and 0.8% cholesterol.

Monkeys were caged individually in thermoregulated rooms. Venous blood samples were drawn after the monkeys were sedated with ketamine HCl (12 mg/kg i.m.). Total cholesterol and triglycerides were determined by the method of Abell28 as modified by the Lipid Research Clinics Protocol.

At the time of study, the monkeys were sedated with...
ketamine (12 mg/kg i.m.) and anesthetized with chloralose (75 mg/kg i.v.). They were intubated and ventilated with room air and supplemental oxygen. Rectal temperature was maintained at 37–38°C with a heating pad.

A catheter (PE 90) was inserted through a femoral artery into the descending aorta for measurement of aortic pressure and to obtain blood samples. Catheters were inserted into both brachial arteries to obtain reference blood samples for determination of blood flow with microspheres. Catheters also were inserted into the femoral and axillary veins for injection of fluids and drugs. A catheter was inserted through a thoracotomy into the left atrium for injection of microspheres. A right thoracotomy also was performed to minimize the movement of the respirator. A ligature around the descending aorta, below the tip of the aortic catheter. The ligature was tightened during hypocapnia and infusion of histamine to prevent hypotension.

Both external carotid arteries were exposed and ligated at the carotid bifurcation. A PE 90 catheter was thinned by stretching and inserted into each lingual artery for infusion of drugs. Serotonin and histamine were infused through the catheters into the common carotid artery.

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

Measurement of Pial Artery Pressure and Cerebral Blood Flow

We have described these methods in cats, rabbits, and rats in detail previously. Pial artery pressure and diameter were measured in an open skull preparation. A craniotomy was performed over the left parietal cortex. A wax dam was built around the cranial window and supported with dental acrylic. The dura was punctured with a needle, resected with ophthalmic scissors, and superfused with artificial cerebrospinal fluid.

Artificial cerebrospinal fluid was prepared as described previously. A mixture of 6.5% CO₂ - 6% O₂ - 87.5% N₂ was bubbled through the solution. This solution was infused through the inlet port at 5–10 ml/min to superfuse the dural opening. We measured pial artery pressure with a micropipette that had a sharp beveled tip 3–6 μm diameter. The micropipette was filled with 1.5 M NaCl solution and pressure was measured with a servonull device (model 4A, Instruments for Physiology and Medicine, Inc., San Diego, CA). A micromanipulator was used to lower the micropipette into the pool of CSF, where it was balanced and a zero value was established. The micropipette then was inserted into the lumen of a pial artery. Pressure was measured in pial arteries 343 ± 45 μm diameter in normal monkeys and 344 ± 39 μm diameter in atherosclerotic monkeys. Diameter of pial arteries was measured with an electronic micrometer (model 142A, ITP, Inc., Sunnyvale, CA), a television camera mounted on a Leitz compound microscope, and a video monitor.

Microspheres 15 μm mean diameter labeled with ⁶⁵Sc, ⁶⁶Sr, ⁶⁸Nb, ¹¹⁵Sn, ¹³¹I, and ¹⁵³Gd (New England Nuclear, Boston, MA) were used to measure cerebral blood flow. Microspheres were injected into the left atrium in 20 seconds. Reference blood samples were withdrawn from the brachial arteries for 10 seconds before until two minutes after injection of spheres. The monkey was sacrificed with KCl i.v. at the end of the experiment. The ipsilateral cerebrum was removed, tissue and blood samples were weighed and counted in a gamma counter, and nuclide separation was accomplished with standard methods. Cerebral blood flow (CBF) was calculated from the equation CBF = (counts/gm of brain x 100 x withdrawal rate of reference blood samples) / counts in the reference blood samples.

We measured blood flow to the entire ipsilateral cerebral hemisphere and 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 that is 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. Blood flow in this region and the remainder of the cerebrum were similar under control conditions and during interventions. We also have measured blood flow to the region exposed by the craniotomy in normal and atherosclerotic monkeys. In 6 normal monkeys, blood flow to cerebrum under the craniotomy in the remainder of the ipsilateral cerebrum differed by only 0.5 ± 3.4 and 2.2 ± 2.8 ml/min × 100 gm during control and intracarotid infusion of serotonin. In 5 atherosclerotic monkeys, blood flow to cerebrum under the craniotomy and in the remainder of the ipsilateral cerebrum differed by 0.9 ± 1.6 and 0.7 ± 2.3 ml/min × 100 gm during control and serotonin. Thus flow to the region under the pial window, and to the area supplied by the punctured pial artery, is similar to flow to the entire hemisphere.

Experimental Protocol

Pial artery pressure and cerebral blood flow were measured using intracarotid infusion of vehicle (0.9% NaCl), histamine, serotonin, and during systemic hypcapnia. We infused 2 and 10 μg/min histamine and 2 and 10 μg/min of serotonin into each common carotid artery, after ligation of the external carotid artery. The volume of infusate was 0.2 ml/min. Hypcapnia was produced by increasing the rate and tidal volume of the respirator. A ligature around the descending aorta was tightened during infusion of histamine and hypcapnia to keep arterial pressure in the upper half of the body from falling. Saline or drugs were infused for at least three minutes before microspheres were injected, and the infusions were continued for two minutes after injection of microspheres. Hypcapnia was maintained for about 15 minutes before microspheres were injected. We waited at least 15 minutes between interventions.
Measurements were made as follows. First, flow and pressure were measured during a control period (intracarotid infusion of vehicle). Second, pial artery pressure was measured during intracarotid infusion of histamine 2 μg/min, but flow was not measured; flow and pressure were measured during infusion of histamine 10 μg/min. Third, flow and pressure were measured during a second control period (infusion of vehicle). Fourth, pial artery pressure was measured during intracarotid infusion of serotonin 2 μg/min, and flow and pressure were measured during infusion of serotonin 10 μg/min. Fifth, flow and pressure were measured during hypocapnia. Thus, cerebral blood flow and pial artery pressure were measured 5 times in each experiment and, in addition, pial artery pressure was measured during infusion of a low dose of histamine and serotonin.

In normal monkeys, arterial PCO₂ was 38 ± 1 mm Hg, PO₂ was 133 ± 8 mmHg, and pH was 7.44 ± 0.02 during control and, except during hypocapnia, they did not change significantly. During hypocapnia, pH increased to 7.71 ± 0.02. In atherosclerotic monkeys, arterial PCO₂ was 39 ± 0.5 mmHg, PO₂ was 127 ± 5 mmHg, and pH was 7.41 ± 0.02 during control, and pH increased to 7.60 ± 0.02 during hypocapnia.

Morphological Studies

The common carotid, internal carotid, and vertebral arteries, and the circle of Willis were removed, examined for gross lesions, and fixed in formalin. Histological study was carried out on paraffin sections of preselected sites from the carotid arteries. Vessels were stained with Hematoxylin-Eosin, Verhoeff-Van Gieson, or occasionally with Oil-Red-O, and morphometric determination was performed with an image analyzer to evaluate area of the lumen, intima, and media, as described previously. Measurements were made in undistended arteries and corrected to estimated values during distention. The approach provides values that are comparable to values obtained when vessels are fixed at in vivo pressure.

Statistical Analysis

Values during interventions were compared with values during the preceding control period using paired t-tests. Unpaired t-tests were used to compare values in normal and atherosclerotic monkeys.

Results

Plasma cholesterol was 105 ± 4.6 mg/dl in normal monkeys and 709 ± 73 mg/dl during the last 3 months of atherogenic diet in atherosclerotic monkeys. Plasma triglycerides were <40 mg/dl in normal and atherosclerotic monkeys.

Morphological Changes

In atherosclerotic monkeys, morphological changes on gross examination were similar to those described previously. The common carotid and internal carotid arteries had pronounced atherosclerotic changes (fig. 1). There was diffuse thickening of the arterial wall by virtually continuous lesions. Histological study of the common carotid arteries demonstrated fibrofatty lesions with necrosis in about 5% of sections and calcification in about 15% of sections. Foam cells were prominent in extracranial internal carotid arteries. Focal areas of medial necrosis were observed. Intracranial arteries usually were normal and lesions, when present, were limited to small fatty streaks.

Morphometric studies were performed to determine the area of the intima, lumen, and media. These studies demonstrated marked increases in intimal area of the common carotid and internal carotid arteries of atherosclerotic monkeys (figs. 2 and 3); in contrast, the intima of normal monkeys is < 0.01 mm². A remarkable finding was that luminal area of the common carotid arteries is virtually normal in atherosclerotic monkeys, despite pronounced proliferation of the intima (fig. 2). Luminal area of the internal carotid artery is moderately reduced in atherosclerotic monkeys (fig. 3). Medial area of atherosclerotic monkeys was preserved in the common carotid arteries and reduced in the internal carotid arteries.

Cerebral Hemodynamics During Control Conditions

Prior to interventions, pressure in pial arteries approximately 350 μm diameter was 22 ± 2 mmHg.
lower than aortic pressure in normal monkeys and 25 ± 3 mmHg lower than aortic pressure in atherosclerotic monkeys (fig. 4). Thus resistance of large arteries (> 350 μm diameter) accounted for more than 25% of total cerebral vascular resistance. This finding supports the concept that large arteries, as well as small vessels, contribute importantly to cerebral vascular resistance in primates, as well as other species.6,7

**Effects of Histamine**

Intracarotid infusion of histamine 10 μg/min increased cerebral blood flow in normal and atherosclerotic monkeys (p > 0.05, atherosclerotic vs. normals) (table 1). Infusion of histamine did not alter the decrease in pressure from the aorta to pial artery.

Thus, histamine produced cerebral vasodilatation in both normal and atherosclerotic monkeys, but it did not affect the drop in pressure from aorta to pial artery. This finding indicates that histamine did not increase the contribution of large arteries to total cerebral vascular resistance.

**Effects of Serotonin**

Intracarotid infusion of serotonin 10 μg/min augmented the decrease in pressure from the aorta to pial artery (figs. 5 and 6). This effect was small in normal monkeys, and larger in atherosclerotic monkeys (table 1). Thus, constriction of large arteries, with reduction in cerebral microvascular pressure, was greater in atherosclerotic monkeys than in normal monkeys. Despite pronounced constriction of large cerebral arteries by serotonin, a decrease in cerebral blood flow was prevented by dilatation of small vessels and presumably by increases in flow through the vertebral arteries.

**Effects of Hypocapnia**

Hyperventilation reduced cerebral blood flow in normal and atherosclerotic monkeys (p > 0.05 vs. normals) (table 1). Hypocapnia did not alter the decrease in pressure from the aorta to pial artery in normal or atherosclerotic monkeys. Thus, hypocapnia produced cerebral vasoconstriction in both normal and atherosclerotic monkeys, but it did not change the contribution of large cerebral arteries to total cerebral vascular resistance.

**Discussion**

There are two major conclusions in this study. First, morphometric studies indicate that, in primates with moderately severe atherosclerosis, the lumen of carotid arteries is relatively well preserved despite marked intimal proliferation. Thus, at this stage of atheroscle-
These findings indicate that moderately severe atherosclerosis produces marked intimal proliferation, and thickening of the carotid arteries, with remarkable preservation of the vascular lumen. Thus, atherosclerotic lesions are displaced outward, with minimal obstruction of the vessels. Similar findings have been made in the coronary and iliac arteries of primates and in coronary arteries of humans. Primates develop atherosclerotic lesions that resemble those that occur in humans. Although humans eventually develop obstructive and occlusive lesions in the carotid arteries, we speculate that marked thickening of the carotid arteries may occur with moderately advanced atherosclerosis, but compensatory mechanisms may allow outward displacement of the lesions with relative preservation of the arterial lumen.

**Hemodynamic Findings**

Serotonin constricts large arteries in several vascular beds, but dilatation of small vessels prevents a decrease in blood flow. We have observed previously that atherosclerosis potentiates constrictor responses of large arteries in the limb more than 10-fold. In this study, serotonin was infused into the carotid arteries to simulate local release of serotonin from platelet aggregates. Serotonin produced a small decrease in cerebral microvascular pressure in normal monkeys, and a large decrease in atherosclerotic monkeys. The decrease in microvascular pressure during infusion of serotonin was the result of constriction of large arteries upstream. Despite constriction of large arteries in the carotid distribution, however, serotonin did not reduce cerebral blood flow. Thus, constriction of large arteries reduced cerebral microvascular pressure, which presumably led to an autoregulatory decrease in resistance of small cerebral vessels, and perhaps also led to an increase in flow through the vertebral arteries to preserve cerebral blood flow. The experimental approach, therefore, allowed detection of augmented vasoconstrictor responses to serotonin in atherosclerotic monkeys that would not have been detected if cerebral blood flow, but not cerebral microvascular pressure, were measured.

In contrast to augmentation of vasoconstrictor responses to serotonin in atherosclerotic monkeys, responses to hypocapnia and histamine were not altered. Several studies indicate that atherosclerosis potentiates constrictor responses to histamine in the coronary circulation and thus may play a role in the pathogenesis of coronary vasospasm. We cannot exclude the possibility that responses to other doses of histamine may be altered in cerebral vessels of atherosclerotic monkeys. Nevertheless, the finding that responses to hypocapnia and histamine are not altered by atherosclerosis suggests that potentiation of vasoconstrictor responses may be somewhat specific for serotonin. It is important to note that aggregation of platelets releases thromboxane and other vasoactive substances, as well as serotonin. It is not known whether atherosclerosis alters cerebral vasoconstrictor responses to these agonists.
Implications

It was surprising to us that moderately severe atherosclerosis produced marked thickening of the vessel wall but compensatory mechanisms allow outward displacement of the lesions, so that the vascular lumen is relatively well preserved. This observation was made in nonhuman primates with moderately severe atherosclerosis, and it is not clear whether carotid arteries of humans manifest similar changes during presymptomatic stages of atherosclerosis.

There is strong, although somewhat indirect, evidence that most TIAs are produced by aggregation of platelets in extracranial arteries. Release of serotonin during platelet aggregation, together with augmented constrictor responses of atherosclerotic cerebral arteries, could contribute to the pathogenesis of TIAs in two ways. First, serotonin might produce enough constriction of large arteries to produce cerebral ischemia. This possibility seems very unlikely. Second, constriction of large arteries by serotonin, in the presence of a subcritical distal stenosis, may contribute to focal cerebral ischemia. Thus, constriction of large arteries, with reduction in cerebral microvascular pressure, could reduce cerebral perfusion pressure and, in the presence of either a fixed stenosis of a distal artery or partial obstruction of a small artery by an embolus, might contribute to focal cerebral ischemia. We speculate, therefore, that release of serotonin during aggregation of platelets in carotid arteries with reduction of cerebral microvascular pressure, may not be a primary cause of TIAs but may nevertheless play a role in the pathogenesis of TIAs.

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