Enhanced Coronary Vasoconstrictive Response to Serotonin Subsides After Removal of Dietary Cholesterol in Atherosclerotic Monkeys

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Abstract Constriction in response to serotonin is enhanced in the coronary arteries of atherosclerotic monkeys. The main objective of the present study was to determine whether abnormal responses to serotonin in atherosclerosis are reversed following removal of dietary cholesterol. In addition, we examined the effect of an atherogenic diet and reduction in dietary cholesterol on vascular responses to activation of ATP-sensitive K⁺ channels with aprikalim. Diameters of small coronary arteries were measured on the epicardial surface of the left ventricle in vivo by using stroboscopic illumination synchronized to the heart cycle to visually freeze the motion of the heart. Diameters were measured with a microscope-video system during topical application of two vasoconstrictor agonists, serotonin and the thromboxane mimetic U46619, and the vasodilator agonists aprikalim and nitroprusside. Responses were compared in normal (n=9), atherosclerotic (n=14; high-cholesterol diet), and regression (n=8; high-cholesterol diet followed by normal diet) monkeys. Constriction of coronary arteries in response to serotonin was enhanced in monkeys on an atherogenic diet and was normal in regression monkeys. Vasoconstriction in response to U46619 and vasodilation in response to nitroprusside and aprikalim were not altered by atherosclerosis. Thus, abnormal vascular responses to serotonin in small coronary arteries of atherosclerotic monkeys without morphological evidence of disease can be reversed to normal by reducing dietary cholesterol.

Key Words • serotonin • U46619 • aprikalim • nitroprusside • coronary resistance

Vascular responsiveness to several agonists is altered by atherosclerosis,1-7 and coronary vasoconstrictor responses to serotonin are augmented in patients with atherosclerosis or unstable angina.5-6 Coronary microvessels relax in response to serotonin in normal monkeys and constrict in atherosclerotic animals.8 Thus, vasomotor abnormalities of atherosclerosis extend to the coronary microcirculation despite an absence of morphological evidence of disease.8-11 A critical question is whether abnormal vascular responses in the atherosclerotic coronary circulation can be restored to normal by reducing dietary cholesterol. Reduction of dietary cholesterol reduces the intimal area of arteries as lipids are reabsorbed from the vessel wall.12-14 Fibrosis progresses within the artery, however, so that maximal vasodilator responses do not improve consistently.15 Nevertheless, studies in the hindlimb and cerebral circulation suggest improvement in vascular function after regression of atherosclerosis, with restoration of endothelium-dependent relaxation15 and reduction in abnormal vasoconstriction in response to serotonin.16,17 It is unclear whether reductions in dietary cholesterol restore vascular reactivity of the coronary circulation to normal. The major objective of the present study was to determine whether reduction of dietary cholesterol in atherosclerotic monkeys abolishes vascular hyperreactivity to serotonin in the coronary circulation.

Activation of ATP-sensitive K⁺ channels may be an important mechanism of vasodilatation and may mediate vascular responses to hypoxia18 and ischemia.19,20 Dilatation of arterioles and the increase in coronary flow during reactive hyperemia are reduced by glibenclamide, which inhibits ATP-sensitive K⁺ channels.21,22 Vasodilation in response to activation of K⁺ channels is reduced in hypertension23 and diabetes24 and may be altered by atherosclerosis. Thus, the second objective of this study was to determine whether atherosclerosis alters the responses of the coronary circulation to activation of ATP-sensitive K⁺ channels with the agonist aprikalim.

Methods

Three groups of adult male cynomolgus monkeys were studied. Normal monkeys (n=9; 6.7±0.7 kg, mean±SEM) were fed commercial laboratory chow (Purina monkey chow, Ralston Purina) and had a plasma cholesterol of 124±9 mg/dL. Atherosclerotic monkeys (n=14; 5.7±0.3 kg) were fed a semipurified atherogenic diet consisting of 20.3% protein, 36.7% carbohydrates, and 43.0% fat for 25±2 months. The cholesterol content of the diet was 682.5 mg per 100 g diet for 0.7% by weight. Atherosclerotic monkey plasma cholesterol was 598±29 mg/dL. Regression monkeys (n=8; 6.4±0.3 kg) were fed an atherogenic diet for 19 months (plasma cholesterol, 640±35 mg/dL) and subsequently a low-cholesterol (normal) diet for 17±3 months (plasma cholesterol, 127±8 mg/dL). For the terminal study monkeys were sedated with ketamine (2 mg/kg IM) and anesthetized with α-chloralose (100 mg/kg)
IV) and urethane (200 mg/kg IV). One femoral and both brachial arteries were cannulated for measurement of arterial pressure and for withdrawal of reference flow samples for measurement of myocardial perfusion. A brachial vein was cannulated for administration of fluids and supplemental anesthetic. Monkeys were ventilated with high-frequency ventilation to minimize respiratory-induced motion and to synchronize motion due to respiration with the heart cycle. High-frequency ventilation was accomplished by introducing a 14-gauge cannula into the trachea caudal to the endotracheal tube and advancing the cannula to the carina. The cannula was connected to a pressure source of 40% O2 and 60% N2. Input pressure was regulated between 4 and 8 psi. Left ventricular dP/dt was used to trigger the opening of a solenoid valve for pressure input for 11 to 25 milliseconds per heart cycle. Arterial blood gases were maintained within physiological limits by varying the input pressure, the duration of ventilation, and positive end-expiratory pressure.

The heart was exposed through a midsternotomy. A solid-state pressure transducer (Millar SF) was inserted into the left ventricle via the carotid artery for measurement of left ventricular dP/dt and pressure. The left atrium was cannulated for injection of radiolabeled microspheres and fluorescein-labeled dextran for illumination of coronary arteries. The epicardial surface of the heart was continuosly suffused with Krebs-Henseleit physiological salt solution (mmol/L: NaCl 118.3, KCl 4.7, CaCl2 2.5, MgSO4 1.2, NaHCO3 25.0, and KH2PO4 1.2) bubbled with 5% CO2, 20% O2, and 75% N2 at 37°C to maintain the physiological environment. Snares were placed around the inferior vena cava and advancing aorta to maintain a constant arterial pressure throughout the study.

Measurement of Coronary Artery Diameter
Epicardial arterial diameters were visualized by using stroboscopic epi-illumination. Briefly, using left ventricular dP/dt as an input into a PDP11/73 microcomputer for the timing of a strobe, the epicardial surface of the beating left ventricle was illuminated for 25 microseconds once per cardiac cycle in late diastole. With this technique the vasculature appeared stationary and was visualized with a microscope (Zeiss) with a Zeiss Neofluor 6.3× objective (numerical aperture, 0.20). The microscope was coupled to a silicon-intensified tube video camera (Dage) and an image-digitizing system (Image Technology). Images of vessels were acquired in nine successive heart cycles and reviewed on a high-resolution monitor; selected images were stored on computer or magnetic tape for later analysis and permanent storage.

Luminal diameters of vessels were acquired during bolus injections of fluorescein-labeled dextran (500 000 MW, 5 mg/mL, 0.1 to 0.3 mL per injection) into the left atrium. Because arteries illuminate before veins, this technique can distinguish between these vessels. Diameters were measured using a digitizing tablet (Summa Graphics). For each intervention, three through six measurements were made of each vessel as the average diameter along a length of the vessel wall. Vessel response was defined as the percent change in diameter from control.

Measurement of Myocardial Perfusion
Myocardial perfusion was measured with the radioactive microsphere technique to assess the stability of the preparation. Since drugs were suffused topically, no change in myocardial perfusion was expected. Briefly, microspheres (1 to 2×106, 15.5 μm in diameter, 7 to 14 μCi/g) labeled with 85Sc, 89Sr, 90Y, 113Sn, 111In, 46Sc, 47Ca, 48Ca, 39K, 89Rb, or 99Nb or 113Cd were vortexed for 5 minutes and injected into the left atrium followed by a saline flush (2 to 3 mL). Prior to and for 90 seconds following injection, two reference flow samples were withdrawn at a constant rate with a pump from both brachial arteries at 1.03 mL/min. At the completion of the experiment the heart was fixed in formaldehyde, and two transmural samples were cut from both the anterior and posterior regions. The transmural pieces were cut into two equal pieces of subepicardium and subendocardium. Tissue and reference flow samples were counted in a germanium crystal counter, and myocardial perfusion was calculated by using standard techniques. Myocardial blood flow was expressed as the weighted mean of all samples from each region.

Morphological Analysis
At the completion of each study a suture was placed around the region of small artery where the diameter was measured. A section of the vessel was removed and fixed in 10% buffered formalin. In each monkey a proximal segment of the left anterior descending coronary artery immediately distal to the left main was also removed, fixed in formalin, and examined for gross atherosclerotic lesions. The vessel samples were processed and embedded in paraffin, and three 8-μm transverse sections of the vessel were prepared for histometric study. Measurements of intimal and medial areas were performed on a single sample from each vessel with an image analyzer. Images were projected at ×60 or ×150, outlined with a cursor, and digitized to arterial area in square millimeters.

Experimental Protocol
Following the surgical preparation, monkeys were allowed to stabilize for 30 minutes during topical suffusion of Krebs’ solution over the artery to be studied. Control diameters and hemodynamics were measured. Myocardial perfusion was measured at the beginning of each drug suffusion to assess the stability of the preparation. Serotonin (10−4 and 10−3 mol/L), U46619 (10−8 and 10−7 mol/L), aprikalim (10−7 and 10−6 mol/L), or nitroprusside (10−5 mol/L) was suffused, and diameter and hemodynamics were measured at steady-state responses (5 to 10 minutes). The order of drug suffusion was randomized.

Source of Chemicals
Serotonin and nitroprusside were obtained from Sigma Chemical Co, U46619 from Cayman Chemical Co, and aprikalim from Rhone-Poulenc Rorer.

Statistical Analysis
Data are presented as mean±SEM. When responses were measured in more than one vessel in an individual monkey the percent change in diameter was averaged, so that n represents the number of vessels. Responses to all vasoactive agents were not measured in all monkeys. Changes in diameter in normal, atherosclerotic, and regression monkeys were compared by ANOVA. When significance was attained (P<.05), individual comparisons were made between groups by using analysis of least squares means corrected for multiple comparisons with the Bonferroni method.

Results
Morphological Changes
Representative illustrations of a proximal segment of the left anterior descending artery and a small coronary artery are shown in Figs 1 and 2. In the large artery of atherosclerotic monkeys there was marked intimal thickening and increased lipid deposition. Intimal area was increased, but medial area was not altered (Fig 3). In regression monkeys, intimal area did not decrease (Figs 1 and 3). In contrast to large arteries, small arteries had minimal changes in intimal and medial layers in atherosclerotic and regression monkeys (Figs 2 and 3).
Responses to Serotonin and U46619

There were no differences in systemic hemodynamics among normal, atherosclerotic, or regression monkeys under control conditions. Before suffusion of serotonin in normal monkeys (n=7), mean arterial pressure was 91±4 mm Hg, heart rate was 171±10 beats per minute (bpm), and myocardial perfusion was 232±35 mL/min×100 g. In atherosclerotic monkeys (n=9), arterial pressure was 85±4 mm Hg, heart rate was 143±8 bpm, and transmural perfusion was 257±24 mL/min×100 g. In regression monkeys (n=6), mean arterial pressure was 85±5 mm Hg, heart rate was 158±7 bpm, and myocardial perfusion was 263±28 mL/min×100 g. Systemic hemodynamics did not change in any group during suffusion of serotonin or other agents.

Serotonin had a minimal effect on the diameter of small arteries in normal monkeys (Fig 4). In contrast, serotonin produced constriction of small arteries in atherosclerotic monkeys (P<.05 versus normal monkeys). In regression monkeys, the constrictor response to serotonin was abolished (P<.05 versus atherosclerotic monkeys).

U46619 produced constriction of small arteries in normal monkeys (Fig 5). Constriction in response to U46619 was similar in normal (n=9), atherosclerotic (n=12), and regression (n=8) monkeys. Thus, altered constrictor responses in atherosclerosis are selective for serotonin.

Response to Aprikalim and Nitroprusside

Aprikalim produced dilation of small arteries in normal monkeys (n=5; Fig 6). Dilation in response to
aprikalim was similar in atherosclerotic monkeys (n=9; Fig 6). Dilation to aprikalim, however, was attenuated in regression monkeys (n=5).

Dilation of small arteries to nitroprusside (10^-5 mol/L) was similar in all groups (percent change from control: normal, 17±5 [n=7]; atherosclerotic, 22±5 [n=9]; and regression, 20±6 [n=5]; P>0.05).

Discussion

The major new finding of the present study is that abnormal vascular responses to serotonin in the coronary circulation of atherosclerotic monkeys can be abolished after reduction of dietary cholesterol. This improvement occurred despite persistence of morphological evidence of significant disease in the large coronary artery. Abnormal responses to serotonin that were observed in small arteries of atherosclerotic monkeys were not associated with morphological evidence of disease in the small arteries. The second new finding in this study was that atherosclerosis does not affect coronary vascular responses to activation of ATP-sensitive K^+ channels. Dilation in response to aprikalim was not impaired in atherosclerotic monkeys.

Critique of Methodology

Several aspects of the techniques used in this study should be considered in relation to interpretation of the results. The technique used to measure responses of the coronary circulation in vivo allows measurements of vessels on the epicardial surface only. Intramyocardial arteries and arterioles cannot be visualized using this technique, and intramyocardial vessel response may be different. Subendocardial arterioles in vitro are more sensitive than epicardial arterioles to some vasodilators such as adenosine or forskolin. Dilation in response to bradykinin, ADP, the calcium ionophore A23187, and nitroprusside was similar in endocardial and epicardial arterioles. In addition, myogenic vasoconstriction is greater in subepicardial than subendocardial arterioles in vivo. Thus, the response of epicardial vessels may not represent the response of vessels throughout the myocardiun.

In the present study control diameters did not differ among the three groups, but they tended to be larger in the regression group than in normal monkeys. The luminal area of large coronary arteries is maintained in atherosclerosis in atherosclerotic monkeys and humans, which suggests vascular "remodeling." Following regression of atherosclerosis, the luminal area of large coronary arteries is increased. The effects of atherosclerosis and regression on the luminal diameter of small arteries similar in size to the vessels in the present study have not been systematically examined. It is possible that the trend toward enlargement of the diameters of small arteries during regression in the present study is similar to previous studies in large arteries.

The response of coronary arteries and arterioles to a variety of pharmacological and physiological stimuli is dependent on the initial diameter of the vessel. Serotonin dilates arterioles less than 100 μm in diameter, but it constricts larger arteries. This trend for larger control diameters in regression monkeys probably does not explain the differences in the response to serotonin. Larger vessels in regression monkeys would favor constriction in response to serotonin. Thus, reduction in vasoconstrictor responses to serotonin in regression monkeys occurred despite a tendency for larger artery size in this group. Furthermore, constriction in response to U46619 was similar in normal, atherosclerotic, and regression monkeys. Thus, the ability of vessels to
constrict did not differ among groups. Because dilation in response to nitroprusside was also similar among groups, the decreased dilation to activation of ATP-sensitive K⁺ channels that we observed in regression monkeys cannot be explained by a nonspecific difference in vasodilator capacity of small arteries.

We considered the possibility that length of time on an atherogenic diet may have influenced the results of this study. The atherosclerotic monkeys consumed the atherogenic diet for 29 months; regression monkeys were on the atherogenic diet for only 18 months before returning to a normal diet. We compared responses to serotonin in a small group of the 18-month atherogenic-diet monkeys with those of the 29-month atherogenic-diet monkeys. The amount of constriction did not differ between the two groups: the percent change in diameter in response to serotonin 10⁻⁶ mol/L was −7±2 short-term and −8±3 long-term; in response to serotonin 10⁻⁵ mol/L, the percent change was −10±4 short-term and −11±3 long-term. Thus, the difference in the amount of constriction between atherosclerotic monkeys and regression monkeys cannot be explained by a difference in the duration of the atherogenic diet.

**Effect of Atherosclerosis and Regression on Coronary Responses to Serotonin**

In animal models of atherosclerosis the responses of large arteries to endothelium-dependent vasodilators and vasoconstrictors are abnormal. Relaxation in response to the endothelium-dependent vasodilators acetylcholine, thrombin, and A23187 is impaired, whereas relaxation to the endothelium-independent vasodilator nitroprusside is unaltered.⁵³–⁵⁶ Vasoconstriction in response to platelet products such as serotonin is augmented.⁵⁷–⁶¹ Several potential mechanisms for altered responses to endothelium-dependent agents in atherosclerosis have been suggested. Basal release and agonist-stimulated production and/or release of endothelium-derived relaxing factor (EDRF) are reduced in atherosclerosis.³²–³⁴ Alternatively, release of nitric oxide may be enhanced in atherosclerosis,³⁴ but degradation of EDRF may also be enhanced,³⁵ resulting in decreased dilation to endothelial-dependent agents. Finally, atherosclerosis may affect availability or utilization of substrate since addition of L-arginine (precursor of EDRF) can restore endothelium-dependent relaxation in both hypercholesterolemia and atherosclerosis toward normal.¹¹,⁶²

Augmented constrictor responses of small arteries to serotonin may involve alterations in synthesis and/or release of EDRF. The response of coronary arteries to serotonin is modulated by release of EDRF through activation of 5HT₁ receptors on endothelium.³⁷–³⁹ Removal of endothelium³⁷–³⁹ or administration of nitro-L-arginine to inhibit production of nitric oxide⁴⁰ enhances constriction of large coronary arteries in response to serotonin via activation of 5HT₂ receptors on vascular smooth muscle. The abnormal response to serotonin could reflect an alteration in the balance between vasodilation through release of EDRF from endothelium (5HT₁) and vasoconstriction through activation of 5HT₂ receptors on smooth muscle. Thus, it is possible that augmented constriction to serotonin in small arteries in atherosclerotic monkeys in the present study is due in part to a diminished release of EDRF.

Augmented constriction in response to serotonin may be related to an interaction between serotonin and neutrophils or platelets in the vessel wall in atherosclerosis.
Effect of Atherosclerosis on Coronary Responses

coronary vasodilation during decreases in perfusion pressure within the autoregulatory range and dilation of collateral and noncollateral vessels during ischemia. During both decreases in perfusion pressure and after coronary occlusion, dilation of arterioles and collaterals less than 100 μm in diameter was inhibited by the ATP-sensitive K⁺ channel inhibitor glibenclamide. In the presence of atherosclerosis and a stenosis of proximal coronary arteries, perfusion pressure downstream from the stenosis is reduced. The ability of downstream vasculature to respond to decreased perfusion pressure and to maintain flow may be mediated in part by activation of ATP-sensitive K⁺ channels. Thus, the ability of the coronary microcirculation to respond to activation of ATP-sensitive K⁺ channels may be especially important in the presence of atherosclerosis.

In the present study we tested the effect of atherosclerosis on activation of ATP-sensitive K⁺ channels by measuring responses of small coronary arteries to aprikalim. Interpretation of the results is dependent on the specificity of aprikalim to activate ATP-sensitive K⁺ channels. Aprikalim selectively activates ATP-sensitive K⁺ channels in cardiac myocytes. Dilation of coronary microvessels in response to aprikalim is inhibited by the ATP-sensitive K⁺ channel inhibitor glibenclamide but not by charybdotoxin or apamin, inhibitors of calcium-activated channels, or inhibitors of nitric oxide synthase. Thus, the magnitude of vasodilation in response to aprikalim is an index of activation of ATP-sensitive K⁺ channels. Vasodilation in response to activation of K⁺ channels is attenuated in both hypertension and diabetes. These findings are in contrast to the present observation that dilation in response to aprikalim was not altered by atherosclerosis. An unexpected finding was a small but significant reduction in dilation in response to aprikalim in regression monkeys. The attenuated response to aprikalim in small arteries in regression animals is not a nonspecific effect since dilation to nitroprusside was not altered.

In summary, the present study demonstrated that abnormal coronary vascular responses to serotonin in atherosclerosis can be abolished by reducing dietary cholesterol. Atherosclerosis does not impair responses of small coronary arteries to activation of ATP-sensitive K⁺ channels. These data may have important implications in treatment of atherosclerosis in the coronary circulation. Evidence that vascular function recovers to normal despite no measurable reductions in lesion size suggests that clinical treatment of hypercholesterolemia and atherosclerosis that achieves only lesion arrest (stabilization) may nonetheless normalize hyperconstrictor responses to serotonin. We speculate that reduction in dietary cholesterol may restore vascular function to normal and reduce the incidence of coronary vasospasm and resulting myocardial ischemia.

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