Role of Vascular Endothelium in Exercise-Induced Dilation of Large Epicardial Coronary Arteries in Conscious Dogs

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Background The role of vascular endothelium in the control of epicardial coronary artery vasomotion during treadmill exercise remains unclear. Therefore, we examined the consequences of in vivo balloon endothelial denudation on external coronary diameter of the left circumflex artery during exercise in conscious dogs.

Methods and Results Seven dogs instrumented for the measurement of arterial blood pressure, external coronary artery diameter, and coronary blood flow were studied during exercise before and up to 21 days after balloon endothelial denudation of the proximal left circumflex artery. Endothelial denudation was confirmed by abolition of the epicardial coronary artery dilation induced by acetylcholine (0.3 µg/kg IV) and reactive hyperemia. Epicardial coronary vasodilatation was observed in the control state during treadmill exercise (+5.2±1.0%). In contrast, a marked vasoconstriction was observed 3 (-4.6±0.6%) and up to 6 days after endothelial denudation. Complete epicardial coronary artery dilation in response to acetylcholine and exercise was restored 9 days after endothelial denudation. In addition, epicardial coronary artery vasomotor responses to acetylcholine and treadmill exercise were closely correlated (r=.82, P<.001). Reactive dilation was not completely restored 21 days after endothelial denudation, but reactive hyperemia and exercise vasomotor responses during the 21 days follow-up were correlated (r=.70, P<.001). Vasodilation induced by nitroglycerin (1 µg/kg IV) was reduced by 25% (P<.01) 3 days after endothelial denudation and returned to its corresponding control level 3 days later. Prazosin (50 µg/kg IV) significantly attenuated the exercise-induced coronary artery constriction after endothelial denudation (+1.5±1.4% versus -4.6±1.0%).

Conclusions These data demonstrate that endothelium is essential for the mediation of epicardial coronary dilation during exercise and may protect these vessels against the vasoconstrictor effect of endogenous catecholamines. (Circulation. 1994;89:2799-2808.)

Key Words • endothelium • denudation • coronary arteries • exercise

Physical exertion, which is associated with adrenergic stimulation and increased circulating catecholamines, is the most common stimulus for myocardial ischemia, and changes in coronary vasomotor tone during sympathetic stimulation influence coronary blood flow.1-4 Indeed, several studies have reported that large epicardial as well as small coronary arteries dilate during exercise in animals5-7 and humans.8-10 Special attention was recently focused on large epicardial coronary arterial responsiveness to exercise because these conductance vessels could play a major role in the pathophysiology of transient myocardial ischemia in patients with atherosclerosis.9,11

Numerous mechanisms may contribute to the dilation of large epicardial coronary vessels during exercise, namely, a passive relaxation due to the increase in coronary arterial pressure, the release of vasoactive substances from the endothelium as the consequence of an increase in coronary blood flow, the activation of coronary artery β-adrenoceptors, and the limitation by the endothelium of the constrictor effects of circulating mediators such as catecholamines or products of aggregating platelets. Some of these mechanisms have been investigated recently through indirect approaches. Exercise-induced increase in coronary blood flow due to the enhancement of myocardial metabolic demand contributes to the dilation of epicardial coronary arteries. Because this dilation is prevented either by a flow-limiting stenosis5 or administration of nitro-l-arginine,12 it appears that exercise-induced increase in coronary blood flow and therefore in shear stress enhances the production of endothelium-derived relaxing factor(s) from the epicardial coronary endothelium. In addition, treadmill exercise promotes the development of cyclic flow variations in conscious dogs with coronary stenosis and endothelial injury, demonstrating that this structure normally prevents platelet activation and subsequent aggregation resulting from the synergism of increased plasma catecholamine levels and the release of proaggregative mediators13,14. Finally, limitation of the constrictor effects of catecholamines by the endothelium has been reported during sympathetic stimulation.15-17

The present study was aimed at directly assessing the role of the vascular endothelium in the control of epicardial coronary artery diameter during treadmill exercise in dogs by performing experiments before and after in vivo balloon endothelial denudation of a large coronary artery. With this technique, initially developed by Chu and Cobb18 and Hayashi et al,19 it was possible
to compare the evolution of the external diameter of a large epicardial coronary artery at rest and during exercise and before and after endothelial denudation, with each dog serving as its own control. In addition, the time course of the functional recovery of the coronary endothelium was investigated by comparing at regular intervals the responses induced by treadmill exercise and those produced by administration of endothelium-dependent (acetylcholine) and -independent (nitroglycerin) coronary vasodilators and by reactive hyperemia (flow-mediated, endothelium-dependent reactive dilation). Finally, the potential contribution of &alpha;1-adrenoceptor stimulation to the vasomotor tone of large epicardial coronary arteries during exercise was also investigated before and after endothelial denudation.

Methods

Surgical Preparation

Sixteen adult mongrel dogs weighing 22 to 30 kg were anesthetized with sodium pentobarbital (30 mg/kg IV), intubated, and ventilated with a respirator. With sterile surgical techniques, a left thoracotomy was performed in the fifth intercostal space, and the heart was suspended in a pericardial cradle. Catheters were implanted in the descending thoracic aorta and in the pulmonary artery. A pair of ultrasonic dimension transducers, 5-MHz piezoelectric crystals (VD 5S, Triton Technology), were attached to Dacron backing and sutured using Ethicon 5-0 suture (Ethicon, Inc) to opposing surfaces of the left circumflex coronary artery 2 to 4 cm from its origin. Care was taken while positioning the transducers to limit dissection of and damage to any visible nerves, and proper alignment of the crystals was confirmed during surgery by monitoring the ultrasonic signal with an oscilloscope. A 10-MHz Doppler flow probe (Crystal Biotech) and, down-stream, an hydraulic occluder (Jones Instruments) were implanted distal to the dimension transducers. In a subgroup of five dogs, a solid-state pressure transducer (model P7A, Konigsberg Instruments, Inc) was introduced into the left ventricle through the apical dimple and secured with purse-string sutures. The pericardium was left open, and all wires and catheters were passed subcutaneously to the back of the dog and brought through the skin between the scapulae. The pneumothorax was evacuated through a chest tube inserted into the sixth intercostal space. Cefazolin (1 g IV) and gentamycin (40 mg IV) were administered before incision and at the end of the surgery. The animal instrumentation and the ensuing experiments were performed in accordance with the official regulations of the French Ministry of Agriculture.

Measurement of Hemodynamic and Coronary Parameters

Aortic pressure was measured with a Statham P231D pressure transducer (Statham Instruments). Left ventricular (LV) pressure and its first derivative (LV dp/dt) were recorded with the Konigsberg gauge. The external diameter of the circumflex coronary artery was measured instantaneously and continuously with an ultrasonic transit-time dimension gauge, and coronary blood flow was measured using a Doppler flowmeter (Triton Technology Inc, System 6, model 200). Left circumflex coronary vascular resistance, which reflects coronary arteriolar tone, was calculated as the ratio of mean arterial pressure to mean coronary blood flow. Data were continuously recorded on a multichannel electrostatic recorder (ES 2000, Gould Instruments Inc).

Experimental Protocols

All experiments were conducted at least 3 weeks after the initial surgery, when the dogs were healthy and apperetic. In 7 of the 16 instrumented dogs, vasodilation of the left circumflex coronary artery in response to administrations of acetylcholine (0.3 &mu;g/kg IV) and nitroglycerin (1 &mu;g/kg IV) were assessed on the first experimental day, with the dog lying quietly on its right side on the experiment table. The drugs were administered in random order through the pulmonary artery catheter. The doses of acetylcholine and nitroglycerin were chosen as those inducing a significant increase in the left circumflex coronary artery diameter at minimal changes in aortic pressure on the basis of previous studies where full dose-response curves were obtained with these vasodilators under the same experimental conditions. In addition, reactive hyperemia following a 20-second coronary occlusion was also assessed. After at least a 3-hour recovery period, the dogs were exercised on a motor-driven treadmill in a protocol involving three successive 3-minute exercise stages at 5, 10, and 12 km/h, respectively (5% slope), as previously described.

The next day, the dogs were lightly anesthetized with 0.5% halothane. Under aseptic conditions, an incision was made to expose the right carotid artery. An 8F left coronary guiding catheter (Schneider-Climo) was inserted through the right carotid artery and positioned in the left coronary ostium under fluoroscopic guidance. A balloon angioplasty catheter (Thru-flex, Medtronic) was inserted through the guiding catheter into the left circumflex coronary artery into the area of the piezoelectric crystals. To avoid the distension of the coronary artery, care was taken to calibrate the balloon catheter according to both the external coronary diameter measured by ultrasonic transit-time dimension gauge and by the estimation of the internal diameter from serial injections of contrast medium (Iopamidol, Schering Laboratories) into the left coronary ostium. Consequently, the size of the balloon was 2.5 or 3 mm. The balloon was inflated with 1 mL air, and the catheter was gently moved back and forth three times over the entire segment from the proximal circumflex artery to the crystal area. This procedure induced endothelial denudation on approximately 2 cm on each side of the crystals, leaving the distal circumflex, the left anterior descending coronary, and the septal arteries intact. The balloon was then deflated, the catheter was withdrawn, and the dogs were allowed to fully recover. Then, the protocols described previously, i.e., acetylcholine and nitroglycerin administrations, reactive hyperemia, and treadmill exercises, were repeated 3, 6, 9, 14, and 21 days after endothelial denudation.

In 9 of the 16 dogs instrumented as previously described, the effects of a continuous infusion of nitroglycerin (0.3 &mu;g·kg⁻¹·min⁻¹ IV) and bolus injection of prazosin (50 &mu;g/kg) on the left circumflex coronary artery diameter were investigated at rest and during treadmill exercise, before and 3 days after endothelial denudation. The 5 dogs that received nitroglycerin were different from the 4 that received prazosin.

Histological Study

Histological study was designed to focus on the immediate lesions induced by balloon denudation. Consequently, 4 additional noninstrumented dogs were killed with a lethal dose of sodium pentobarbital immediately after balloon denudation performed in the same conditions as those used for instrumented dogs. For analysis by light microscopy, segments about 10 mm long were taken from the circumflex coronary artery at the site of balloon denudation and from the proximal left anterior descending coronary artery serving as control. Samples were fixed in phosphate-buffered formaldehyde (pH 7.2) and processed for paraffin embedding. Serial sections 5 &mu;m thick were routinely stained with hematoxylin phloxin saffron for overall analysis, orcein picro indigo carmin for staining of elastic fibers, and picropolymorphic dye for staining of collagen fibers. Analysis was completed by immunostaining of fibronectin (rabbit polyclonal anti-fibronectin: DAKO A245 1/100).
Data Analysis

Data were read from the strip-charts under baseline conditions and at peak increases in coronary diameter for the various drugs. Reactive hyperemic responses are reported as peak-to-baseline increases in epicardial coronary diameter and repayment-to-debt ratios of coronary blood flow. The tracings corresponding to the responses to reactive hyperemia after the release of a 20-second coronary occlusion were digitized using a Hewlett-Packard scanner interfaced to a Macintosh computer. The area-under-the-curve representing the volume and duration of the coronary blood flow deficit during coronary arterial occlusion, ie, the flow debt, and the excess of coronary blood flow that followed the release of the coronary artery occlusion, ie, the flow repayment, were quantified with an image analysis software. During exercises, the measurements of heart rate, arterial and LV pressures, epicardial coronary diameter, and coronary blood flow were averaged over the last 30 seconds of each 3-minute exercise stage.

Statistical Analysis

Values are given as mean±SEM. Comparisons of the coronary artery diameter responses between (1) acetylcholine and nitroglycerin and (2) acetylcholine and reactive hyperemia measured before and at the five times after endothelial denudation were made with a two-way ANCOVA for repeated measures, using basal epicardial coronary diameter as the independent variable. For each treatment, the difference between the epicardial coronary artery vasomotor response observed at each time after endothelial denudation and the vasomotor response observed before endothelial denudation was tested by contrast analysis.

Comparisons between coronary artery responses measured during treadmill exercise before and at the five times after endothelial denudation were performed by a one-way ANCOVA for repeated measures using basal epicardial coronary diameter as the independent variable. The differences between dilation observed at each time after endothelial denudation and dilation recorded before endothelial denudation were performed by contrast analysis.

Concerning the other hemodynamic parameters, the responses were analyzed using a two-way ANOVA for repeated measures. Correlations were performed using the linear regression method. The statistical analyses were performed on a PC compatible computer using BMDP statistical software (BMDP). A value of P<.05 was considered statistically significant.

Results

Effects of Endothelial Denudation on Basal Parameters

Experiments were initiated in seven dogs. Baseline coronary and hemodynamic parameters values at rest, before and after endothelial denudation, are reported in Table 1. Throughout the experiments, none of these parameters changed significantly except the epicardial coronary artery diameter, which increased by 6.9±2.2% (P<.001) and 4.5±2.7% (P<.01) from 344±246 to 366±231 μm at 3 and 6 days after endothelial denudation, respectively.

Effects of Endothelial Denudation on Hemodynamic and Coronary Responses to Exercise

Table 1 summarizes the systemic and coronary hemodynamic responses to exercise, before and after endothelial denudation, and Fig 1 illustrates the changes in coronary artery diameter at the different levels of exercise. Before endothelial denudation, heart rate, LV systolic and mean aortic pressures, LV dP/dt, coronary blood flow, and coronary artery diameter increased and coronary resistance decreased at each level of exercise. Up to 21 days after endothelial denudation, responses to exercise of LV and systemic hemodynamics, coronary blood flow, and coronary resistance remained unchanged. The normal exercise-induced dilation of large epicardial coronary artery was, however, abolished 3 and 6 days after endothelial denudation and reverted to marked constriction of the vessel (−4.6±0.6% from 3665±231 to −2.2±1.3% from 3574±215 μm, respectively; P<.001). Restoration of a significant exercise-induced dilation of large epicardial coronary artery was observed 9 days after endothelial denudation, and this response remained unchanged up to 21 days.

Effects of Endothelial Denudation on Coronary Responses to Acetylcholine, Nitroglycerin, and Reactive Hyperemia

Table 2 summarizes the effects of acetylcholine and nitroglycerin administration and of reactive hyperemia on coronary artery diameter and vascular resistance before and after endothelial denudation.

Before endothelial denudation, acetylcholine and nitroglycerin significantly decreased coronary resistance (−58.7±5.9% and −34.6±5.2%, respectively) and increased coronary artery diameter (4.4±0.7% and 6.3±0.5%, respectively). After endothelial denudation, the effects of acetylcholine and nitroglycerin on coronary resistance remained unaffected. Responses to nitroglycerin in the deendothelialized segment were unaffected, except 3 days after endothelial denudation where the dilation was reduced by 25% (P<.001). The increase in coronary artery diameter induced by nitroglycerin was restored at day 6 and was significantly greater than before endothelial denudation at days 14 (P<.01) and 21 (P<.05). Acetylcholine-induced increase in coronary artery diameter was reduced by 92% and 75% compared with the control response 3 and 6 days after endothelial denudation, respectively (P<.001). Thereafter, this response was completely restored. Finally, when all data obtained before and after endothelial denudation were pooled together, epicardial coronary artery vasomotor responses to acetylcholine and to treadmill exercise were closely correlated (r=.82, P<.001) (Fig 2).

Before endothelial denudation, reactive hyperemia was associated with a 278±32% increase in coronary blood flow followed by a delayed increase in coronary artery diameter that reached 4.2±1.3%. Coronary blood flow repayment-to-debt ratios remained comparable throughout the study. Three days after endothelial denudation and despite a similar increase in coronary blood flow (269±19%), the increase in coronary artery diameter was abolished. Because of technical problems (hydraulic occluder disruptions), the complete time course recovery of reactive hyperemia was available in four of the seven dogs during the 21-day follow-up. As shown in Table 2, complete restoration of reactive dilation was not observed up to 21 days after endothelial denudation. In these 4 dogs, reactive hyperemia and exercise vasomotor responses were correlated (r=.70, P<.001). In addition, when the results of reactive hyperemia vasomotor response available for the seven dogs were pooled, reactive hyperemia and exercise vasomotor responses were correlated (r=.62, P<.001).
TABLE 1. Absolute Values of Hemodynamic and Coronary Parameters at Rest and at the Maximal Treadmill Exercise Levels Before and at Different Days After Endothelial Denudation

<table>
<thead>
<tr>
<th></th>
<th>Before Endothelium Denudation</th>
<th>Days After Endothelium Denudation</th>
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<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>HR, bpm (n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>96±4</td>
<td>96±2</td>
</tr>
<tr>
<td>Change at Ex 12</td>
<td>+119±10</td>
<td>+118±7</td>
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<tr>
<td>Mean AP, mm Hg (n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>95±4</td>
<td>95±3</td>
</tr>
<tr>
<td>Change at Ex 12</td>
<td>+19±4</td>
<td>+25±5</td>
</tr>
<tr>
<td>Mean CBF, cm/s (n=7)</td>
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<td></td>
</tr>
<tr>
<td>Rest</td>
<td>15.3±2.7</td>
<td>16.4±3.4</td>
</tr>
<tr>
<td>Change at Ex 12</td>
<td>+20.9±5.5</td>
<td>+24.1±7.4</td>
</tr>
<tr>
<td>Mean CVR, U (n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>7.5±1.3</td>
<td>7.6±1.5</td>
</tr>
<tr>
<td>Change at Ex 12</td>
<td>−3.6±0.6</td>
<td>−2.9±1.0</td>
</tr>
<tr>
<td>Mean CD, μm (n=7)</td>
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<td></td>
</tr>
<tr>
<td>Rest</td>
<td>3444±246</td>
<td>3665±231†</td>
</tr>
<tr>
<td>Change at Ex 12</td>
<td>+173±35</td>
<td>−174±27†</td>
</tr>
<tr>
<td>LVP, mm Hg (n=5)</td>
<td></td>
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<tr>
<td>Rest</td>
<td>128±4</td>
<td>132±3</td>
</tr>
<tr>
<td>Change at Ex 12</td>
<td>+38±7</td>
<td>+41±5</td>
</tr>
<tr>
<td>LV dP/dt, mm Hg/s (n=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>2990±76</td>
<td>2885±193</td>
</tr>
<tr>
<td>Change at Ex 12</td>
<td>+3226±319</td>
<td>+3467±313</td>
</tr>
</tbody>
</table>

HR indicates heart rate, bpm, beats per minute; AP, arterial blood pressure; CBF, coronary blood flow; CVR, coronary vascular resistance; CD, external coronary diameter; LVP, left ventricular pressure; and Ex 12, peak exercise at 12 km/h. Values are given as mean±SEM. *P<.01, †P<.001, significantly different from corresponding value before endothelial denudation.

Effects of Nitroglycerin Infusion on Coronary Dynamics Before and After Endothelial Denudation

Because it was possible that the initial 200-μm increase in resting coronary artery diameter induced by endothelial denudation could partly account for the observed constriction of the artery during exercise, basal coronary diameter of the circumflex artery was artificially increased by approximately 200 μm using a constant intravenous infusion of nitroglycerin (0.3 μg · kg⁻¹ · min⁻¹) in five additional dogs. Before endothelial denudation, nitroglycerin increased the resting coronary artery diameter by 205±57 μm from 3075±333 μm and remained at this level throughout the exercise (Fig 3, top). In contrast, 3 days after endothelial denudation, while nitroglycerin increased the resting coronary artery diameter (+115±36 μm from 3363±306 μm), exercise still induced a vasoconstriction (−154±47 μm at 12 km/h from a resting value of 3478±308 μm, P<.01) that was similar to that observed after saline administration (−156±39 μm at 12 km/h for a resting value of 3328±284 μm) (Fig 3, bottom).

Effects of Prazosin on Coronary Dynamics Before and After Endothelial Denudation

Because α₁-adrenoceptor stimulation could contribute to the vasoconstriction observed after endothelial denudation during exercise, the effects of prazosin were examined in four additional dogs. Before endothelial denudation, prazosin significantly increased the resting coronary artery diameter (+79±27 μm from 3024±326 μm, P<.01), which further increased during exercise.
TABLE 2. Baseline and Peak Changes in Absolute Values of Coronary Hemodynamic Parameters After Acetylcholine and Nitroglycerin Administrations and Reactive Hyperemia Before and at Different Days After Endothelial Denudation

<table>
<thead>
<tr>
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<th>Before Endothelial Denudation</th>
<th>Days After Endothelial Denudation</th>
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<tr>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Acetylcholine, 0.3 μg/kg (n=7)</td>
<td></td>
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</tr>
<tr>
<td>Mean CD, μm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3429±251</td>
<td>3643±229†</td>
</tr>
<tr>
<td>Change from baseline</td>
<td>+147±25</td>
<td>+10±3‡</td>
</tr>
<tr>
<td>Mean CVR, U</td>
<td></td>
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<tr>
<td>Baseline</td>
<td>8.2±1.8</td>
<td>7.2±1.7</td>
</tr>
<tr>
<td>Change from baseline</td>
<td>-4.4±0.6</td>
<td>-4.5±1.2</td>
</tr>
<tr>
<td>Nitroglycerin, 1 μg/kg (n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CD, μm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3445±248</td>
<td>3646±230†</td>
</tr>
<tr>
<td>Change from baseline</td>
<td>+211±18</td>
<td>+164±28‡</td>
</tr>
<tr>
<td>Mean CVR, U</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>10.2±2.6</td>
<td>7.1±1.5</td>
</tr>
<tr>
<td>Change from baseline</td>
<td>-3.4±1.0</td>
<td>-1.8±0.7</td>
</tr>
<tr>
<td>Reactive Hyperemia (n=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CD, μm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3330±426</td>
<td>3606±416†</td>
</tr>
<tr>
<td>Change from baseline</td>
<td>+124±9</td>
<td>0‡</td>
</tr>
<tr>
<td>Change in CBF</td>
<td>3.32±0.80</td>
<td>3.74±0.64</td>
</tr>
</tbody>
</table>

CD indicates external coronary diameter; CVR, coronary vascular resistance; and CBF, coronary blood flow. Values are given as mean±SEM. *P<.05, †P<.01, ‡P<.001, significantly different from corresponding value before endothelial denudation.

(+174±53 μm at 12 km/h from a resting value of 3103±314 μm) (Fig 4, top). Three days after endothelial denudation, in the absence of prazosin, exercise induced a marked coronary artery constriction (−153±33 μm at 12 km/h from a resting value of 3354±300 μm, P<.01). After prazosin administration, coronary artery diameter increased at rest by 86±71 μm from 3293±288 μm (P<.01) and remained increased during exercise (Fig 4, bottom). Thus, prazosin alleviated the exercise-induced epicardial coronary artery constriction observed normally 3 days after endothelial denudation.

Histology of the Coronary Artery After Endothelial Denudation

As shown in Fig 5, balloon denudation induced a total or subtotal loss of the endothelial coating. Endothelial shedding was associated with foci of internal elastic fibers disruption as underlined by orcein dye. Moderate exudates together with red blood cells were sometimes spreading on the bare intima. The picropolychromic stain showing light cyanophilic material indicated the integrity of the basement membrane, which was confirmed by fibronectin immunolabeling.

Discussion

The results of the present investigation demonstrate that in conscious dogs, the vasodilation of epicardial coronary artery normally observed during exercise is converted to a vasoconstriction after mechanical endothelium removal, the recovery of a normal epicardial vasodilation following exercise occurs within 9 days after endothelial denudation and is correlated with the responsiveness of the vessel to both acetylcholine and reactive hyperemia, and the coronary endothelium plays a protective role against α1-adrenoceptor-medi-
ate constrictions of epicardial coronary arteries during exercise. Despite accumulating evidence that endothelial cells are of major importance in the integration of regional vasomotion during physiological and/or pharmacological stimuli, few attempts have been made to assess the function of this structure during exercise in conscious animals. Physical exercise induces sympathetic activation with an increase in both cardiac sympathetic nerve activity and release of circulating catecholamines. The result is an increase in myocardial oxygen demand through changes in heart rate and contractility, which is adequately matched under normal conditions by an augmented oxygen supply after metabolic dilation of small resistance and large epicardial conductance coronary arteries. Previous studies have indirectly suggested that the endothelium contributes to a large extent to the exercise-induced dilation of epicardial coronary arteries through a flow-mediated release of endothelium-derived relaxing factors. Other studies have also shown that changes in coronary blood flow and dilation of epicardial coronary arteries during exercise are governed by the competing forces of direct adrenoceptor-mediated coronary vasoconstriction and metabolic vasodilation, i.e., blood flow-mediated dilation. Finally, a pivotal role of the endothelium for limiting adrenergic vasoconstriction has also been demonstrated.

To our knowledge, the present study is the first one that investigates in conscious animals the role of the coronary endothelium in the vasodilatory response of conductance vessels to exercise by a direct approach, i.e., by mechanically removing the epicardial coronary endothelium. As we showed previously, this technique results in a local deendothelialization of the proximal site of the circumflex coronary artery 3 days after denudation, as illustrated by a complete loss of responsiveness of the denuded vessel to acetylcholine and reactive hyperemia during the week after denudation in vivo and to acetylcholine in vitro. In agreement with several other studies, we also confirm here by histological findings that this balloon denudation technique results in immediate and successful local removal of the coronary endothelium.

The major finding of the present study is that the loss of the dilating response of the left circumflex coronary artery to acetylcholine and reactive dilation (two well-recognized indexes of the integrity of a functional coronary endothelium) is associated with exercise-induced vasoconstriction of this artery during the first
week after endothelial denudation. In addition, we observed simultaneous recovery of normal epicardial coronary artery vasodilator responses to acetylcholine and exercise 9 days after denudation. Finally, despite a delayed pattern of recovery for reactive dilation after endothelial denudation, vasomotor responses to both acetylcholine and reactive hyperemia were correlated to those induced by exercise. In the present study, coronary blood flow and calculated coronary vascular resistance were not significantly different at baseline or on any of the other study days, nor did either of these variables change significantly with exercise at any of the study points. However, coronary diameters were different at baseline and in response to exercise on the days immediately after endothelial denudation. This apparent discrepancy between size, flow, and resistance of the coronary arteries, also reported by Chu and Cobb, can be explained by the fact that large coronary vessels contribute only slightly to total coronary vascular resistance in normal conscious dogs and that our mechanical endothelial denudation procedure was limited to the area of measurement of the left circumflex coronary artery diameter. In addition, although direct measurements of myocardial oxygen consumption were not possible, the primary determinants of myocardial oxygen consumption, ie, heart rate, myocardial contractility, and aortic pressure, were monitored continuously throughout the exercise periods and remained within the same range before and after denudation. Finally, the different exercise tests were performed under comparable increases in coronary perfusion pressure, and it is unlikely that passive dilation of large coronary arteries has contributed to the results. Thus, our data tend to demonstrate that the endothelium is the major factor involved in the modulation of large epicardial coronary artery dilation during exercise in conscious dogs.

Several of the mechanisms by which the endothelium mediates epicardial coronary artery vasodilation during exercise have been previously addressed. The role of blood flow–mediated dilation has been emphasized by Schwartz et al, who demonstrated that a flow-limiting stenosis prevented the exercise-induced dilation of a large epicardial coronary artery proximal to the stenosis. This result suggests that exercise-induced increase in coronary blood flow and therefore in shear stress enhances the production of endothelium-derived relaxing factor(s), and it is recognized that epicardial coronary artery dilation in response to the increase in blood flow after temporary coronary artery occlusion is mediated through an endothelium-dependent mechanism. This concept is in agreement with the recent study of Wang et al, who reported that vasodilation of a large...
coronary artery during treadmill exercise is reverted to vasoconstriction after administration of nitro-L-arginine. In the present study, there is a close correlation between the vasomotor responses to acetylcholine and exercise, demonstrating that a functional endothelium is essential for the mediation of epicardial coronary artery dilation during exercise. In addition, the observation of a correlation between reactive dilation and exercise argues for the concept that vasodilation of epicardial coronary arteries during exercise is in part mediated through a flow-dependent mechanism. However, another important finding in the present study is that the recovery of epicardial coronary artery dilation in response to reactive hyperemia is notably delayed compared with that of the responses to acetylcholine and exercise. Reasons for this difference are still unclear, but similar discrepancies between flow-mediated coronary responses to vasodilator agents and sympathetic stimuli have been reported in human coronary arteries. Although the increases in coronary artery diameter resulting from an increase in coronary blood flow during exercise and reactive hyperemia are both endothelium-dependent responses, their mechanisms might differ. For example, it has been clearly demonstrated that the production of nitric oxide from endothelial cells is the principal mediator responsible for the dilation of the circumflex coronary artery during exercise, and the complete and concomitant restoration of epicardial coronary artery dilation in response to both acetylcholine and exercise 9 days after endothelial denudation in the present study is in agreement with such a mechanism. In contrast, inhibition of nitric oxide production by l-arginine analogues does not completely abolish reactive dilation in response to a transient coronary occlusion, suggesting that mediators other than nitric oxide might be involved in reactive dilation at the level of large coronary arteries. Indeed, the increases in coronary blood flow and therefore in shear stress resulting from dynamic exercise and reactive hyperemia are different in many aspects (eg, changes in heart rate, myocardial contractility and/or relaxation, coronary perfusion pressure), and resulting flow-induced increase in coronary artery diameter is certainly not identical in these two situations. Analysis of these mechanisms was not in the scope of this study, but it must be underlined that if reactive dilation was not completely restored in magnitude to its control level 21 days after endothelial denudation, this response progressively recovered between days 9 and 21, suggesting that restoration of normal reactive dilation requires a complete recovery of endothelial cells both in their amount and in their architecture, whereas the same requirements may not necessarily be as constraining for acetylcholine and exercise-induced increase in coronary artery diameter. In agreement with this hypothesis, a previous study conducted in an experimental model similar to ours found that 5 weeks after mechanical endothelial denudation, coronary segments from injured regions still exhibited cells that were not polygonal in appearance and showed an abnormal axial orientation. Because flow-mediated dilation is closely linked to the elongated shape of endothelial cells and their orientation in the direction of blood flow within the vessel, it is possible that the same phenomenon also occurred in the present study, and this could account for the delayed recovery of the responses to reactive dilation compared with that of the responses to acetylcholine and exercise. Finally, in contrast with the findings of Shimokawa et al, we did not observe any delayed impairment of endothelium- and receptor-dependent responses in the region of regenerated endothelial cells. Differences in species and experimental design may account for these divergent results.

Other mechanisms could be proposed for the epicardial coronary artery constriction observed during exercise after endothelial denudation. Among these mechanisms, we paid special attention to $\alpha_1$ -adrenoceptors that play a major role in the control of large coronary vessel tone during exercise in conscious dogs. Although it was not possible to infuse prazosin directly within the coronary artery, we found that prazosin alleviated the exercise-induced epicardial coronary artery constriction usually observed 3 days after endothelial denudation. Thus, it is clear that mechanical removal of the endothelium unmasks the vasoconstrictor effects of exercise-induced catecholamine release at the level of epicardial coronary $\alpha_1$-adrenoceptors, an effect that can be prevented by prazosin administration. This result further emphasizes the fundamental role of the coronary endothelium as a protective structure against the deleterious effect of vasoactive mediators released during exercise.

**Study Limitations**

The current balloon endothelium denudation technique has been described and considered an adequate model of in vivo deendothelialization. In the present study, we did not perform serial histological analysis to further characterize the relations between recovery of a functional response and endothelium regeneration. However, this point was addressed previously, and it has been shown that restoration of the responses to acetylcholine and reactive dilation may be attributed to the regeneration of a functionally active endothelium. Although balloon denudation did not alter basal systemic or coronary hemodynamics, despite the great care taken in choosing the balloon size, a slight although significant increase in resting coronary artery diameter of about 200 μm was observed. Given the importance of the basal release of endothelium-derived relaxing factors in maintaining a permanent vasodilator tone at the level of large epicardial coronary arteries in conscious dogs, endothelium denudation would have been expected to induce a vasoconstriction. However, such a postdenudation increase in coronary artery diameter has been reported by others and is probably due to a passive distension of the vessel during balloon inflation rather than to deendothelialization per se. In addition, Joly et al have shown that in vivo balloon inflation of rat carotid arteries stimulated the production of nitric oxide originating from vascular smooth muscle cells through induction of nitric oxide synthase activity by interleukin-1β, a cytokine released from blood cells at sites of vascular injury. However, this phenomenon probably is not of major importance in the present experimental model of local and controlled coronary balloon deendothelialization because we previously demonstrated that administration of nitro-L-arginine methyl ester does not alter the observed increase in resting coronary artery diameter 2 to 4 days after endothelial denudation performed with the same technique.
We also observed a slight but significant decrease in the response of the circumflex coronary artery to nitroglycerin 3 days after endothelial denudation. Vascular relaxation to nitrates is a widely accepted index of smooth muscle integrity after endothelial denudation, and a similar depression of the response to nitroglycerin has been previously reported by other investigators using this technique. This could at least in part be the consequence of the increase in the basal diameter of the artery, which would tend to limit the vasodilatory capacity of the vessel. It is also likely that moderate lesions of the internal elastic lamina and inner smooth muscle layers contribute to this slight and transient reduction of the response to nitroglycerin in the present study. Conversely, the significant hyperrelaxation to nitroglycerin that we observed 14 to 21 days after endothelial denudation could also be related to smooth muscle cell proliferation, as previously reported.

To further investigate the extent to which the initial increase in circumflex coronary artery diameter could contribute to the observed constriction of the vessel during exercise after endothelial denudation, we increased the basal coronary artery diameter by approximately 200 μm through a continuous infusion of nitroglycerin. Although exercise induced significant dilation of the epicardial coronary artery before denudation, constriction of the vessel still occurred after denudation, despite the simultaneous infusion of nitroglycerin at the same dose. This indicates that the initial increase in coronary artery diameter after mechanical endothelium removal is not the primary cause of the observed constriction of the large coronary vessels. Clearly, this exercise-induced vasoconstriction is closely linked to the multiple events occurring during the exercise itself since it was not prevented by nitroglycerin despite a similar significant increase in the resting coronary artery diameter after endothelial denudation.

**Clinical Implications**

In patients with angina, activation of the sympathetic nervous system is an important trigger mechanism for the development of ischemia. Furthermore, exercise is the most common clinical situation leading to myocardial ischemia. It has been demonstrated that endothelium-dependent vasodilator function is lost in a progressive manner during the early stages of atherosclerosis with various endothelium-dependent stimuli affected in a hierarchical fashion. In this regard, it has been shown that atherosclerotic coronary arteries constrict during exercise and the cold pressor test, with each stimulus increasing sympathetic activity and coronary blood flow. In addition, Vita et al. showed that the endothelial dysfunction that characterizes early and late atherosclerosis is associated with a marked increase in sensitivity to the constrictor effects of catecholamines. The present study highlights these findings by demonstrating that endothelium modulates the vasomotor response of a large epicardial coronary artery during exercise and emphasizes the role of catecholamines in mediating exercise-induced vasoconstriction as suggested by Vita et al. The pathological consequences of paradoxical vasoconstriction of epicardial coronary arteries during exercise are poorly known. In the present animal model, the vasoconstriction of epicardial coronary artery did not affect the increase in coronary blood flow during exercise. However, endothelial dysfunction was limited to a short coronary artery segment, which contrasts with diffuse alteration of endothelial function observed in atherosclerotic patients. In addition, the uncoupling of resistance vessel tone to metabolic factors may represent an important mechanism through which disturbances in endothelial function in atherosclerosis may lead to development of myocardial ischemia. In patients with coronary stenosis, however, the role of paradoxical epicardial coronary artery constriction during exercise in mediating myocardial ischemia is not similar to that in our experimental model as, in contrast with the clinical data reported by Gage et al., nitroglycerin infusion was not potent enough in the present study to suppress the exercise-induced epicardial coronary artery constriction.

**Conclusions**

This study demonstrates that after selective endothelial denudation, the normal exercise-induced coronary vasodilation is replaced by a constriction of large epicardial coronary arteries. This phenomenon is reversible since the recovery of a normal epicardial vasodilation after exercise occurs within 9 days after endothelial denudation and is correlated with that of the responsiveness of the vessel to both acetylcholine and reactive hyperemia. Endothelium thus is essential for the mediation of epicardial coronary artery dilation in response to an exercise-induced increase in coronary blood flow, and it protects the vessel against the vasoconstrictor effect of catecholamines released during exercise. These mechanisms could play a role in the incidence of myocardial ischemia in patients with coronary endothelial dysfunction.

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