Endothelial Modulation of the Coronary Vasculature in Vessels Perfused via Mature Collaterals

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Previous in vivo studies have shown that vasopressin, which releases the endothelium-derived relaxing factor and constricts coronary smooth muscle, produces augmented constriction of coronary microvessels perfused by mature collaterals. We hypothesized that chronic perfusion through collaterals produces endothelial dysfunction in the recipient vasculature. Mature collaterals were stimulated in mongrel dogs by the ameroid constrictor technique. After 3–6 months, rings of conduit vessels (obtuse marginal) were studied in organ chambers, and coronary microvessels (100–220 µm) were studied in a pressurized, no-flow state with a microvessel imaging apparatus. Eleven dogs were used as controls. Large vessels were preconstricted with prostaglandin F₂α to 30–70% of the maximum potassium chloride tension, and microvessels were preconstricted to 20–60% of the baseline diameter with the thromboxane mimic U46619. Relaxations to the receptor-mediated agents acetylcholine and ADP were markedly impaired in collateral-dependent coronary microvessels, whereas relaxations to nitroglycerin were enhanced compared with microvessels from control dogs. Relaxation to the calcium ionophore A23187, which releases the endothelium-derived relaxing factor through nonreceptor-mediated mechanisms, were similar in control and ameroid microvessels. Constriction to vasopressin was augmented in collateral-dependent microvessels compared with controls. Responses to all agonists were similar between control and collateral-dependent large vascular rings. In conclusion, chronic perfusion through collateral vessels selectively impairs receptor-mediated endothelium-dependent relaxations and augments constriction to vasopressin in the coronary microcirculation. These findings may have important implications regarding neurohumoral regulation of perfusion to collateral-dependent myocardium. (Circulation 1990;81:1938–1947)

After occlusion of a major coronary artery, the only blood flow to the ischemic myocardium arrives via coronary collaterals.1,2 If coronary occlusion develops gradually during weeks or months, the collateral vasculature enlarges sufficiently to prevent myocardial infarction. Well-developed coronary collaterals provide normal perfusion to the recipient myocardium during rest and exercise.3 Despite this, vasomotor control of these vessels appears to vary substantially from that of the innate coronary vasculature.4,5 Specifically, these vessels respond minimally to α-adrenergic stimuli4 and are hyperresponsive to the vasoconstrictor effects of vasopressin.4,6 More recently, we have found that arterioles within the myocardium perfused by collaterals exhibit augmented constriction to vasopressin in vivo.6 Vasopressin produces vasoconstriction via a direct action on vascular smooth muscle7–10 and elicits the release of the endothelium-derived relaxing factor.11,12 This dual effect of vasopressin is shared by several neurohumoral substances such as serotonin,13 acetylcholine,14 and norepinephrine.15 Thus, one explanation for augmented constriction of arterioles to vasopressin in the collateral-dependent myocardium is that chronic perfusion through collateral vessels alters endothelial regulation of arteriolar tone. The purpose of the present study was to test
this hypothesis. We employed an in vitro approach to allow examination of both larger conduit arteries and coronary resistance vessels in collagen perfused myocardium. Responses to several neurohumoral and pharmacologic substances that cause vascular relaxation via the endothelium and to nitroglycerin, which causes vascular relaxation by a direct action on the vascular smooth muscle, were examined.

**Methods**

**Production of Mature Collateral Vessels**

Eight adult mongrel dogs of either sex were anesthetized initially with thiopental (30 mg/kg i.v.) followed by pentobarbital sodium (30 mg/kg). By sterile techniques, a left thoracotomy was performed through the fourth intercostal space during mechanical ventilation. The pericardium was opened, and an ameroid occluder of either 2.77 or 3.0 mm in diameter was placed around the proximal left circumflex coronary artery. The pericardium was loosely approximated, and the thoracotomy was anatomically closed. The dogs were allowed to recover. Experiments were performed 3–6 months after ameroid occluder placement. Eleven normal dogs were used as controls.

**Experimental Preparation**

On the day of the study, the dogs were anesthetized with pentobarbital and thiopental, intubated, and mechanically ventilated. A left thoracotomy was performed through the fourth intercostal space. Cannulas were placed in the femoral artery and veins. Arterial blood gases were frequently checked, and the rate and depth of mechanical ventilation were adjusted to maintain these within physiological range. The pericardium was opened, and the aorta and circumflex coronary artery just distal to the ameroid occluder were cannulated. Simultaneous aortic and circumflex coronary artery pressures were then recorded. Subsequently, the heart was rapidly excised and placed in cold Kreb's buffer with the composition (mM): NaCl 118.3, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, and glucose 11.1. The area of the ameroid occluder was examined visually, and circumflex coronary artery occlusion was confirmed.

**Large Coronary Artery Studies**

Epicardial obtuse marginal branches of the circumflex coronary artery were dissected free and cut into 1–3-mm rings. These were studied in 25 ml organ chambers containing Kreb's buffer aerated with 95% O₂-5% CO₂ maintained at 37°C. Each ring was suspended on two stainless-steel clips passed through the lumen. One clip was anchored inside the organ chamber, and the other was connected with 4.0 silk suture to a force transducer (Grass FT03C). Changes in isometric force were recorded with a direct-writing recorder (Gould Inc., Cleveland, Ohio) coupled to a bridge amplifier. The rings were placed at the optimum point in the length-tension relation for force generation, as determined by responses to 100 mM KCl and determined for each ring before starting an experiment.

**Microvessel Studies**

Microvessel studies from the same animals were carefully dissected from obtuse marginal branches of the circumflex coronary artery with an ×40 dissecting microscope. These segments varied from 100 to 220 μm in diameter and 2 to 3 mm in length. They were placed in a Plexiglas isolated organ chamber, cannulated with dual glass micropipettes measuring 50–100 μm in diameter, and secured with 10-0 nylon monofilament suture. Kreb's buffer was continuously circulated through the organ chamber. The response of microvessels to KCl (100 mM) was determined at various distending pressures ranging from 5 to 40 mm Hg. The maximum response was found to occur with a distending pressure of 20 mm Hg. The vessels were then pressurized to 20 mm Hg in a no-flow state with a manometer filled with Kreb's buffer. Using a split-screen Leitz microscope (×100) connected to a Hitachi video camera, the vessel image was projected onto a TV monitor. A video electronic dimension analyzer (Living Systems Instrumentation, Burlington, Vermont) was used to measure lumen diameter and wall thickness. A pressure transducer measured distending pressure via a side arm cannula positional immediately proximal to one of the micropipettes. Measurements were recorded with a Gould RS3000 strip-chart recorder. The vessels were allowed to bathe in the organ chamber for 60–120 minutes before each intervention.

**Study Protocols**

Large coronary arteries. After equilibration, the vascular rings were preconstricted with prostaglandin (PG) F₂₀ (30–70% maximum potassium chloride response). One or two dose-responses were performed with each ring. Increasing concentrations of either acetylcholine, ADP, calcium ionophore A23187, or nitroglycerin were added to the bath. In addition, the response of vasopressin on nonpreconstricted vessels was examined.

Microvessels. After microvessels were preconstricted with 0.1–10 μM U46619 (a stable thromboxane A₂ analog) by 20–60% of the resting baseline diameter, either acetylcholine, ADP, nitroglycerin, or the calcium ionophore A23187 was applied extrauminally. In addition, vasopressin was applied to minimally preconstricted vessels (<10% of a resting baseline diameter). After the maximum dose of vasopressin (1,000 μU/ml) was administered, 10⁻⁷ M hemoglobin was added to the bath. Because hemoglobin selectively inactivates the endothelium-derived relaxing factor (EDRF), the additional constriction that occurred after hemoglobin provided insight into the amount of EDRF released in response to vasopressin. The order of drug administration was randomized for acetylcholine, ADP, vasopressin, and nitroglycerin. Responses to the calcium ionophore A23187 were always examined last. Ves-
sels were washed three times with Kreb's buffer and allowed to equilibrate for 15 minutes between interventions. From one to four interventions were performed on each vessel, and two or three microvessels from each animal were examined. To determine if thoracotomy or instrumentation affected endothelium-dependent responses, microvessels (two) arising from diagonal branches of the left anterior descending coronary artery (LAD) were dissected from amered dogs, and responses to nitroglycerin and acetylcholine were examined.

Endothelial function. To directly assess the role of the endothelium in mediating responses to acetylcholine, the endothelium was selectively damaged in six dogs by infusing 10 \( \mu \)g/ml saponin for 30 seconds into the proximal LAD while occluding the artery proximally.\(^{18}\) The occlusion was released after infusion of saponin. The heart was subsequently excised, and microvessels from the diagonal branches of the LAD, and the obtuse marginal branches of the circumflex coronary artery were dissected and mounted in the microvessel-imaging apparatus. Responses to acetylcholine and nitroprusside were examined as in the previously described protocol.

Drugs. Acetylcholine hydrogen chloride, ADP (from equine muscle), bradykinin, calcium ionophore A23187, bovine hemoglobin, sodium nitroprusside, and saponin were obtained from Sigma Chemical (St. Louis, Missouri). Nitroglycerin was obtained from Du Pont Pharmaceuticals (Wilmington, Delaware). U46619 and PGF\(_{2\alpha}\) were obtained from Upjohn (Kalamazoo, Michigan). Vasopressin was obtained from Parke-Davis (Morris Plains, New Jersey). All drugs except saponin were dissolved in distilled water; saponin was dissolved in Kreb's buffer. Calcium ionophore A23187 solutions were mixed in DMSO to make a 10\(^{-3}\) M stock solution and stored at \(-20^\circ\) C. A stock solution of U46619 (10\(^{-3}\) M) was made in distilled water and stored at \(-20^\circ\) C. All dilutions were prepared daily. Hemoglobin solutions were prepared before each experiment by dissolving 6.45 g free bovine hemoglobin in 100 ml distilled water containing 0.174 mg sodium dithionite. These solutions were dialyzed for 3 hours against distilled water.

Data Analysis

For microvessels, relaxations were expressed as a percent change in the preconstricted diameter. In large vascular rings, data were expressed as percent relaxation of the preconstricted tension. ED\(_{50}\) and mean responses at each dose of each drug were compared by analysis of variance. Whenever significance was indicated, Scheffé's \( F \) test for multiple comparisons was used to compare groups. Because coronary microvessel constrictions to vasopressin were not normally distributed, the nonparametric rank-sum test was used to compare normal and amered groups. Values were expressed as mean±SEM. A \( p \) value of less than 0.05 was considered significant.

Results

In dogs with circumflex amered occlusion, the mean aortic pressure was 109±8 mm Hg, and the circumflex coronary artery pressure (distal to the amered occluder) was 88±9 mm Hg. Thus, the average pressure difference across the collateral vasculature was 21±3 mm Hg in these animals (\( p < 0.05 \)). This value for peripheral coronary pressure is approximately fivefold to sixfold that observed in dogs with unstimulated collaterals.\(^{19}\)

The mean microvessel diameter was 179±9 \( \mu \)m in control dogs and 163±10 \( \mu \)m in amered dogs. The degree of preconstriction of coronary microvessels was similar in control and amered groups (35±4% vs. 33±5%, respectively).

Rings of obtuse marginal branches of the circumflex coronary artery measured 1–3 mm in diameter. The resting tension was 3.0±0.2 in the control group and 3.8±0.3 in the amered group. The mean PG F\(_{2\alpha}\)-induced tension was 3.4±0.3 g in the control group and 3.0±0.4 g in the amered group.

Nitroglycerin

Relaxation of coronary microvessels in response to nitroglycerin was significantly greater in the amered group compared with the control group (Figure 2A). Large vessels from amered and control dogs relaxed in an identical fashion to nitroglycerin (Figure 2B).

Endothelium-Dependent Responses

Relaxations of coronary microvessels induced by the receptor-mediated agents acetylcholine and
ADP were significantly reduced in microvessels from collateral-dependent myocardium compared with the control group (Figures 3A and 4A, respectively).

In rings of large coronary arteries, relaxations induced by either acetylcholine (Figure 3B) or ADP (Figure 4B) were identical to those of rings from control dogs.

The calcium ionophore A23187, which releases EDRF through a nonreceptor-mediated mechanism, produced nearly identical relaxation in ameroid and control groups, both in coronary
Figure 4. Plots of responses of coronary microvessels (100–200 μm) (panel A) and rings (1–3 mm) (panel B) from obtuse marginal branches of the left circumflex artery to ADP in control (six) and ameroid (six) dogs in vitro. Microvessels were preconstricted by 20–60% of the baseline diameter with U46619, and rings were preconstricted by 30–70% of the maximum potassium chloride–induced tension with prostaglandin F₂α. Responses are percent relaxation of the preconstricted diameter (panel A) or tension (panel B). *p<0.05, **p<0.001.

microvessels (Figure 5A) and in rings from large coronary vessels (Figure 5B).

Vasopressin

Coronary microvessels from collateral-dependent myocardium constricted 33±14% in response to vasopressin (1,000 μU/ml) compared with 5±2% in control microvessels (Figure 6A). The addition of 10 μM hemoglobin, which inactivates EDRF, increased the constriction an additional 12±4% in the ameroid microvessels and 21±6% in control microvessels. The total constriction of vasopressin and hemoglobin was

Figure 5. Plots of responses of coronary microvessels (100–220 μm) (panel A) and rings (1–3 mm) (panel B) from obtuse marginal branches of the left circumflex artery to the calcium ionophore A23187 in control (six) and ameroid (six) dogs in vitro. Microvessels were preconstricted by 20–60% of the baseline diameter with U46619, and rings were preconstricted by 30–70% or the maximum potassium chloride–induced tension with prostaglandin F₂α. Responses are percent relaxation of the preconstricted diameter (panel A) or tension (panel B).
similar between the two groups of microvessels. Rings from large coronary arteries of ameroid dogs showed minimal constriction to vasopressin (1,000 μU/min), whereas no constriction was observed in rings from control dogs (Figure 6B).

Effect of Endothelial Damage
The intraluminal infusion of saponin abolished relaxations of coronary microvessels to acetylcholine (Figure 7A) while not altering responses to the endothelium-independent agent sodium nitroprusside (Figure 7B).

Effect of Thoracotomy and Ameroid Occluder Placement
Relaxations of coronary microvessels to either acetylcholine or nitroglycerin from the LAD distri-
distribution in ameroïd dogs (two) and the circumflex coronary artery distribution of control dogs (six) were identical (Figure 8).

**Discussion**

The present study demonstrates that endothelial modulation of coronary microvessel tone is impaired in microvessels of the collateral-dependent myocardium. Relaxations to the receptor-dependent agonists acetylcholine and ADP were reduced, and vasoconstriction to vasopressin substantially enhanced. Importantly, relaxations to the calcium ionophore A23187, which elicits the release of the EDRF via receptor-independent mechanisms, were not altered. Despite these findings in microvessels chronically perfused by coronary collaterals, endothelium-dependent vascular relaxations of larger conduit vessels removed from this region were not altered. These studies demonstrate that chronic coronary occlusion with subsequent longstanding perfusion through mature coronary collaterals may impair endothelial regulation of the vascular smooth muscle in the coronary microcirculation.

These findings provide insight to our previous observation that vasopressin appeared to not only produce marked constriction of mature collaterals but also cause enhanced constriction of arterioles within the collateral-dependent myocardium. In the coronary microvessels, vasopressin causes vascular constriction via a direct action on vascular smooth muscle that is in part attenuated by the concomitant release of EDRF. It is therefore reasonable to assume that disorders that produce endothelial dysfunction might enhance constriction to vasopressin. In the present study, such an impairment in endothelial function was documented in collateral-perfused coronary microvessels by reduced relaxations to acetylcholine and ADP. In concert with these observations, constrictions to vasopressin in vitro were found to be substantially enhanced. Several other neurohumoral agents also exert such a dual effect on the coronary vasculature, including serotonin, acetylcholine, and norepinephrine. It is interesting to speculate that these agents may exert a potentiated vasoconstrictor effect in coronary microvessels within collateral-dependent myocardium.

The enhanced constriction of collateral-dependent microvessels to vasopressin may have been related to endothelial dysfunction or smooth muscle hypercontractility. To gain insight into this probability, hemoglobin was added to selectively inactivate the endothelium-derived constricting factor. The total constriction resulting from vasopressin and hemoglobin was not statistically different in normal and collateral-perfused microvessels, although it tended to be greater in the collateral-perfused vessels. This observation suggests that the predominant reason for enhanced constriction to vasopressin is related to decreased release of EDRF, although some contribution of smooth muscle hypercontractility cannot be excluded.

In the present study, endothelium-dependent vascular relaxation to the calcium ionophore A23187 was found to be normal with coronary microvessels of the collateral-dependent myocardium, although responses to acetylcholine and ADP were diminished. The calcium ionophore A23187 elicits the release of the EDRF, presumably by causing influx of calcium into the endothelial cells and thereby bypassing receptor and second-messenger mechanisms. Acetylcholine and ADP release EDRF by interacting with muscarinic and purinergic receptors, respectively. Such a discrepancy between the effects of a disease state on receptor- and nonreceptor-mediated EDRF release has been observed in some studies of atherosclerotic vessels but not in others. There

**FIGURE 8.** Plots of responses of microvessels (100–220 μm) from control (nonameroïd) obtuse marginal branches of the left circumflex artery (six) and branches of the left anterior descending artery in ameroïd dogs (two). Microvessels were preconstricted to 20–60% of the baseline diameter with U46619. Acetylcholine or nitroglycerin was administered in incremental doses. Responses are percent relaxation of the preconstricted diameter.
are two potential explanations for this finding. One is that chronic perfusion through coronary collaterals selectively decreases either endothelial cell membrane receptor affinity, number, or interaction with second-messenger systems while not altering the biosynthetic pathway, release, or degradation of EDRF. A second is that the EDRF released in response to the calcium ionophore A23187 is a different compound from that released in response to either acetylcholine or ADP. This latter possibility is supported by recent studies from our laboratory in which the release of nitric oxide and related compounds from the vascular endothelium of porcine coronary arteries was measured by chemiluminescence.24 These studies showed that release of EDRF both under basal conditions and in response to the calcium ionophore A23187 is associated with the release of nitric oxide (detected by chemiluminescence) or a compound that is degraded to nitric oxide in a strong reducing environment. In contrast, during release of EDRF in response to the receptor-mediated agents bradykinin and ADP, release of nitric oxide (in excess of the basal release) could not be detected. It is therefore conceivable that at least two EDRFs exist, one released basally and in response to the calcium ionophore A23187 and a second released in response to muscarinic and purinergic receptor activation, and that the biosynthesis, release, or degradation of the latter is altered in the coronary microcirculation chronically perfused via mature coronary collaterals. The EDRF produces vascular relaxation, predominantly by activating vascular smooth muscle guanyl-
ate cyclase.\textsuperscript{25} It is unlikely that decreased sensitivity of guanylate cyclase with the coronary microcirculation of collateral-dependent myocardium could account for the present findings. Relaxations to glycerin (which also activates guanylate cyclase via its active metabolites) were not diminished in these vessels but in fact were slightly yet significantly enhanced. The reason for this enhanced relaxation to glycerin is unclear but may relate to the diminished endogenous release of EDRF. Inhibition of EDRF may acutely augment relaxations to nitrovasodilators.\textsuperscript{26,27} This is presumably related to removal of the basal influence of EDRF on vascular smooth muscle guanylate cyclase and consequent sensitization of the enzyme.

In the present study, responses of coronary microvessels from the circumflex collateral-dependent myocardium were compared with coronary microvessels removed from the circumflex region of myocardium of animals without collaterals. Thus, changes in endothelial function cannot be attributed to regional differences (LAD vs. circumflex) in coronary microvessel function. It is also unlikely that the differences observed in the present study were related to prior thoracotomy or circumflex instrumentation. Studies performed on LAD microvessels removed from dogs with collaterals demonstrated normal endothelium-dependent vascular relaxations. Furthermore, responses of the larger conduit vessels were not altered in animals with mature collaterals. Thus, it cannot be concluded that prior thoracotomy or circumflex instrumentation per se caused a global impairment in coronary endothelium-dependent vascular relaxation.

Underlying Mechanisms

It is unclear why endothelial regulation of vascular smooth muscle is impaired in collateral-dependent myocardium. One possibility is that during the period of ameroid occlusion (usually 10–21 days), the collateral vasculature fails to develop at a rate sufficiently rapid to prevent a period of myocardial and vascular ischemia. Vascular ischemia caused by transient coronary occlusion may acutely impair coronary endothelium-dependent vascular relaxation.\textsuperscript{28,29} It has recently been suggested that after endothelial injury, endothelial function may be chronically impaired despite endothelial regeneration.\textsuperscript{30} It is thus conceivable that the endothelium was rendered ischemic during the period of ameroid occlusion and that this resulted in prolonged endothelial dysfunction. This phenomenon may also occur in humans who develop collaterals in response to gradual coronary atherosclerotic occlusion.

In an effort to detect morphological abnormalities of the microvascular endothelium in collateral-perfused myocardium, a segment of collateral-dependent myocardium was fix-perfused with glutaraldehyde and subsequently examined by transmission electron microscopy (Figure 9). In this preliminary experiment, we were unable to identify gross abnormalities of the collateral-dependent microvascular endothelium. Specifically, regions of endothelial denudation were not observed, and the endothelium appeared morphologically normal, unlike that reported for regenerated endothelium.\textsuperscript{30} This preliminary observation suggests that the arteriolar endothelium was not rendered ischemic during ameroid closure (and subsequent regeneration) but that other aspects of collateral growth or perfusion may alter endothelial function.

A second possibility is that the production or release of EDRF may be regulated by the ambient pressure within the vasculature. Such a phenomenon has been suggested in studies by Miller et al.,\textsuperscript{31} who have shown that the endothelium-dependent vascular relaxation is enhanced in veins that have been transplanted into the arterial circulation. The pressure distal to the collateral vessels is usually substantially reduced compared with that in the aorta (in the present study, averaging 21 mm Hg less). While this pressure gradient may seem small, it was probably greater during collateral development. In addition, the pulsatile nature of flow may be altered distal to developing collaterals. Alterations of the pulsatile nature of flow may influence EDRF synthesis.\textsuperscript{32}

Finally, it is conceivable that during collateral growth, angiogenic factors are released that may cause proliferation of endothelial cells within the coronary microvessels of the recipient myocardium. Proliferating endothelial cells may lose several functional characteristics, possibly including the ability to synthesize and release EDRF.

Implications

Mature coronary collaterals offer only minimal resistance to coronary perfusion and when fully developed permit normal perfusion both at rest and during exercise.\textsuperscript{3} Regulation of perfusion to collateral-dependent myocardium occurs predominantly at the level of resistance vessels. The present study shows that endothelial regulation of vascular smooth muscle within these vessels is abnormal 3–6 months after the onset of collateral development. These findings suggest that neurohumoral regulation of perfusion to collateral-dependent myocardium may be importantly altered in myocardium, resulting in impaired dilatation to some agents and enhanced constriction to others.

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References


24. Guerra R Jr, Myers PR, Harrison DG: Endothelium-derived relaxing factor (EDRF) and nitric oxide (NO) release from intact vessels under baseline and stimulated conditions. FASEB J 1989;3:A1144


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