Reactivity of Canine Isolated Epicardial Collateral Coronary Arteries

Relation to Vessel Structure

J.A. Angus, J.E. Ward, J.J. Smolich, and G.A. McPherson

To study the relation between structure and vascular reactivity in mature coronary collateral arteries, we prepared 17 dogs with a casein occluder near the origin of the circumflex coronary artery. At least 24 weeks later, we examined the reactivity of surface collateral arteries (—500 μm i.d.) to a range of constrictor and dilator agents and compared them with normal left anterior descending coronary arteries of similar size branching away from the collateral zone. Pairs of normal and collateral arteries 2 mm long were mounted in a double-vessel myograph for isometric force recording. Arteries were contracted by K+ (124 mM) or by cumulative addition of endothelin-1 (1–100 nM) or U46619 (1–300 nM), a thromboxane A2 mimetic drug. In each case, the collateral vessels contracted to approximately half the force generated by the normal arteries. When partially contracted by K+ (25–30 mM), the collateral vessels had a greater range of relaxation and similar sensitivity to acetylcholine, sodium nitroprusside, and cromakalim compared with normal arteries. Morphological and morphometric analyses revealed that the collateral arteries had thickened adventitia, thinner media, ruptured internal elastic laminae, and a thick neointima lined by endothelium. Theoretical calculations of luminal area were made for isotonic conditions in response to constrictor stimuli. Despite the poor contractility of the collateral arteries, the neointimal luminal encroachment further reduced the lumen to zero, an exaggerated response compared with normal arteries. Coronary collateral arteries are thus compromised flow conduits that may play a role in vasospastic angina. (Circulation Research 1991;69:1340–1352)

Oclusion of a major coronary artery induces the development of a collateral circulation, which supplies the metabolic requirements of potentially ischemic myocardium beyond the occlusion.1 Epicardial collateral arteries arise from native, thin-walled, narrow channels. After the major artery occlusion, these vessels rapidly increase their luminal diameter and undergo substantial, long-term structural remodeling.2,3 After this remodeling, the collateral vessels have the morphological appearance of small arteries but display several characteristic features. These features include a ruptured internal elastic lamina separating inner longitudinal and outer circular smooth muscle layers, as well as a variable degree of subintimal thickening.2–4 The effect of these structural features on the passive properties of collateral arteries has, to our knowledge, not been evaluated.

Given these differences in vessel structure, studies have addressed whether the pharmacological reactivity of these vessels differs from that of normal coronary arteries of similar diameter. In the larger tortuous epicardial collateral arteries, radioligand binding studies showed no difference in β1- or β2-adrenoceptor number or affinity,5 whereas in isolated vascular ring preparations of these surface collateral vessels, α-adrenoceptors appeared to be absent. In agreement with these findings, transcollateral resistance, in vivo, was unaltered by methoxamine or clonidine.6 Of potential interest, the collateral vessels appeared to be more reactive to vasopressin than normal arteries both in vivo7 and in vitro.6 Recent experiments have shown that small (100–200-μm) microvessels serving collateral-dependent myocardium also contracted more strongly to vasopressin compared with controls.8 These vessels had markedly impaired relaxation responses to acetylcholine and ADP but normal and enhanced relaxation responses

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to the calcium ionophore A23187 and nitroglycerin, respectively.

In the present study, we have compared the reactivity of three classes of coronary artery of comparable internal diameter: 1) mature epicardial collateral arteries, 2) normal coronary arteries, and 3) pseudo-normal epicardial arteries (taken from a branch distal to the occluder) from dogs 6 months or more after implantation of casein occluders. Our goal was to examine in the in vitro reactivity of these vessels to vasopressin, endothelin-1 (a potent vasoconstrictor peptide released from endothelium),\(^8\) and the thromboxane A\(_2\) mimetic U46619, the latter two being potentially important, locally generated vasoactive substances. Given the reported abnormal endothelial cell response to acetylcholine in the collateral microvessels,\(^6\) we also examined the response to acetylcholine and sodium nitroprusside in these epicardial collateral arteries. In addition, we tested the hypothesis that collateral vessels are more reactive than normal vasculature to the K\(^+\) channel opener cromakalim, as demonstrated in hypoxic rat skeletal muscle\(^10\) and in collateralized hearts in anesthetized dogs.\(^11\)

Finally, we determined the resting membrane potential and examined the vessel morphology to aid our interpretation of the functional myograph studies. The combination of isometric myographic results and morphologic data was used to predict how these collateral arteries may behave to alter blood flow in vivo.

**Materials and Methods**

**Surgical Procedures**

*Group 1.* Anesthesia was induced in eight mongrel dogs (15–29 kg) with thiopentone sodium (20 mg/kg) and in nine greyhounds (20–35 kg) with a mixture of ketamine (5 mg/kg) and xylazine (2 mg/kg). The dogs were intubated, and anesthesia was maintained by ventilation with a combination of halothane, nitrous oxide, and oxygen. Under sterile conditions, a thoracotomy was performed in the fourth or fifth left intercostal space. The pericardium was incised over the left atrial appendix, and a snug-fitting casein constrictor enclosed in a stainless-steel sleeve was placed around the proximal part of the left circumflex coronary artery (CCA). The diameter of the central lumen of the constrictors used in mongrel dogs was 2.5 or 2.8 mm (Three Point Products, Montreal, Canada); in greyhounds, the diameter was 3.2, or 3.5 mm (occluders were constructed at the Baker Medical Research Institute, Melbourne, Australia). The pericardial incision was closed, and the thoracotomy was repaired. After the operation, dogs received pethidine hydrochloride (50 mg i.m.), as necessary for pain relief. Antibiotics were given during the operation (1 g i.v. floroxacin sodium) and after the operation for 7 days (250 mg amoxycillin three times daily).

At least 24 weeks after implantation of the casein constrictor, mongrel and greyhound dogs were deeply anesthetized with Diprivan (5 mg/kg i.v., propofol, ICT, Australia) and \(\alpha\)-chloralose (80–200 mg/kg i.v.). After exposure through a left thoracotomy, the heart was rapidly removed and placed in ice-cold Krebs' physiological salt solution (PSS; for composition, see below) saturated with 5% CO\(_2\) in O\(_2\). The left and right ventricular free walls were pinned out flat in a dish with the bottom covered with Silastic rubber (Sylgard Silastic, Dow Corning, Midland, Mich.) and immersed in cold PSS. The surface collateral vessels between the left anterior descending coronary artery (LAD) and the CCA were easily visualized and carefully dissected free with the aid of a zoom stereomicroscope (×20, Olympus SZT) (Figure 1). Approximately similar-sized arteries from the LAD branching toward the right ventricle were removed as control arteries (Figure 1). In four greyhounds, arteries were also removed from small branches of the CCA distal to the occluder but branching away from the LAD. These were termed "pseudo-normal" arteries. Generally two samples (2–3 mm long) of each vessel type were removed and mounted in myograph chambers.

*Group 2.* A separate group of four normal, unoperated greyhound dogs were anesthetized with Diprivan and \(\alpha\)-chloralose as for the group 1 dogs, and the hearts were removed. From each of these four hearts, two normal arteries were removed from branches of the LAD coursing toward the CCA. These vessels were mounted in separate myographs to determine the relaxation concentration–response curves to the range of vasodilator stimuli in the presence of either high (40 mM) or low (25–30 mM) K\(^+\) solution.

**Pressure Measurements**

In five anesthetized greyhound dogs (from group 1), coronary arterial pressures were simultaneously measured in the LAD and CCA beds before harvesting vessels for the myograph. Teflon catheters were placed in a side branch of the LAD and a marginal branch of the CCA distal to the casein occluder. Phasic and mean arterial pressures were recorded from CDX-11 pressure transducers (Cobe, Colo.). Average pressures were recorded over several minutes.

**Myograph**

Arteries were mounted in the myograph as 2-mm-long ring segments on 40-μm-diameter stainless steel wires in cold PSS of the following composition (mM): NaCl 119, KCl 4.7, KH\(_2\)PO\(_4\) 1.18, MgSO\(_4\) 1.17, NaHCO\(_3\) 25, CaCl\(_2\) 2.5, EDTA 0.026, and glucose 5.5 saturated with 5% CO\(_2\) in O\(_2\), pH 7.4. The mounting and normalization procedure for small arteries has been described previously.\(^12\) In brief, two vessels were mounted in each myograph, one normal and one collateral or one normal and one pseudonormal. Up to three myographs (J.P. Trading, Aarhus, Denmark) were used on the one day; the third myograph was enclosed in a Faraday cage for electrophysiological studies. In all group data calculations, only the results from one of each type of artery from each dog was
used. After mounting and equilibration at 37°C, the vessels were subjected to a routine procedure to determine the passive length-tension curve. The artery was stretched in small steps every minute until the equivalent transmural pressure \( P \) (where \( P = \text{wall tension/interval radius} \)) was just over 100 mm Hg. A normalization computer program determined the vessel diameter at 100 mm Hg \((D_{100})\) and calculated the appropriate micrometer reading to set the artery at a passive stretch appropriate for a diameter of \(0.9D_{100}\) \((D_1)\). This level of stretch is approximately the point where maximum active force is developed. The equivalent transmural pressure \( P_1 \) at \( D_1 \) was calculated \((P_1 = T_1/r_1, \text{where } T_1 \text{ is tension and } r_1 \text{ is the radius})\) and gives a measure of the equivalence of passive stretch for vessels of different lumen diameter.

**Electrophysiology**

The resting membrane potentials of 11 normal and 14 collateral vessels were assessed using conventional glass microelectrodes. The microelectrodes were made from filaments of glass. The microelectrodes were filled with 0.5 M KCl to give tip resistances of \(\sim 100 \text{ M}\Omega\). The reference electrodes were similarly prepared and positioned close to the impaling electrode to minimize the stimulus artifact. The electrodes were placed in Ag/AgCl holders (World Precision Instruments) and connected to a differential amplifier (model 160, Baker Medical Research Institute, Melbourne, Australia). The electrodes were advanced using a pneumatic manipulator (Clark Electromedical Instruments, Pangbourne, UK) mounted on a micromanipulator (Prior Co. Ltd., Bishop’s Stortford, UK) to impale cells within vessels mounted on the myograph and measure their membrane potential. The myograph was mounted within a screened cage (Baker Medical Research Institute) on an air bed (Micro-g, Technical Manufacturing Co., Woburn, Mass.). The membrane potential was recorded on a dual-beam storage oscilloscope (model 5113, Tektronix, Beaverton, Ore.) and on a dual-channel chart recorder (W&W, Basel, Switzerland).

**Protocol**

After mounting and normalization, the control and collateral vessels were activated with K⁺ depolarizing salt solution (KSS, 124 mM K⁺). After washout, cumulative concentration-contraction curves were obtained for endothelin-1 and U46619, a thromboxane \(A_2\) mimetic, with at least 30 minutes between agents. In some experiments other agonists were tested, including serotonin and methoxamine. Vasoressin was tested in greyhound arteries only.

To test vasodilator agents, the arteries were contracted submaximally with 25–35 mM K⁺. After a
stable contraction had been achieved, cumulative additions of acetylcholine or sodium nitroprusside were made. In some experiments, the relaxation response to the endothelium-dependent e2-adrenoreceptor agonist UK14304 was tested. Greyhound arteries only were also tested with the K+ channel opener cromakalim (relaxation) in K+-precontracted vessels. In each case, the PSS was replaced three times with drug-free solution, and a 30-minute rest period was allowed between curves before recontracting the artery with K+. Pseudonormal arteries were tested for contraction to endothelin-1, U46619, and KSS and for relaxation to acetylcholine, sodium nitroprusside, and cromakalim.

In a separate group of greyhound normal coronary arteries (group 2), a series of relaxation curves to acetylcholine, sodium nitroprusside, and cromakalim were constructed in vessels precontracted with low (25–30 mM) or high (40 mM) K+.

**Morphology**

At the end of each myograph experiment, the passive stretch on each artery was reduced to zero. The vessels were then removed from the myographs and fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer. After removal of the mounting wires, the tissues were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer, dehydrated in acetone, and embedded in Epon 812. One normal, one collagenal, and sometimes one pseudonormal vessel were cut into 2-μm-thick transverse sections and stained with toluidine blue.

One section from each artery was projected from a microscope (Neo-Promar projection microscope, Leitz, Wetzlar, FRG) onto a digitizing tablet (Complot, 7000 Digitizer, Bausch & Lomb, Austin, Tex.). The tablet was attached to an Olivetti M28 computer (Ivrea, Italy). A digitizing computer program (MEASURE, Capricorn Scientific Software) was used to calculate various dimensions. A light-pen stylus was used to trace the outlines of the projected cross section of the artery corresponding to the circumferential outline of the adventitia, the adventitial/medial border, and the medial/endothelial border in normal arteries. In collateral arteries, the medial/neointimal border and the inner luminal circumference were measured. In the computer program, the lines traced were digitized every 0.5 mm, and the values were stored on an x–y array. The area enclosed within the outline was calculated by "Green's theorem in the plane," a standard applied mathematics formula for calculating irregular areas. From these regions, the luminal area, the area enclosed by the adventitial/medial border, and the medial area were calculated. From these area measurements, the radii of various elements were calculated, assuming a circular vessel.

**Casting of the Coronary Vasculature**

One mongrel heart (29 kg, 40 weeks after constrictor implantation) and one greyhound heart (35 kg, 63 weeks after constrictor implantation) were perfused in situ via a cannula in the aortic root with saline followed by 10% buffered formalin after arresting the heart with KCl (1 M). A previously published technique was used for adequate fixation of the myocardium at a perfusion pressure of 100 mm Hg. Through the same cannula, 30–50 μl well-mixed methacrylate (dental acrylic, Vertimex, Holland) was injected by hand and allowed to set hard. The heart was removed from the chest and suspended in a beaker of saturated KOH. After 1 week and two changes of the KOH solution, the acrylic cast was removed, washed, and photographed (Figure 1).

**Data Analysis and Statistics**

Increases in wall tension in response to vasoconstrictors were considered as active pressure changes (ΔP), where ΔP = wall tension/radius at D1 (mN/mm² units were converted to mm Hg units). For vasodilator agents, the responses were calculated as percentage of the range of contraction to the K+ depolarization. Values of ΔP or percent relaxation were also averaged at given concentrations of agents. For acetylcholine curves in greyhound vessels and for curves to sodium nitroprusside in mongrels, average sensitivity (EC50) was calculated from the EC50 values defined from the individual logistic-fitted concentration-normalized response curves. For all other concentration–response curves, the data sets were incomplete because a plateau maximum response could not be determined; therefore, no EC50 value could be estimated. When curves could not be statistically fitted within the group, the estimate was abandoned for that group to avoid any "subset" estimations of EC50. Comparisons between data from mongrel and greyhound hearts were made by two-way analysis of variance (ANOVA) with split plot. Student's unpaired (two-tailed) t test was used to compare EC50 values or maximum/minimum values of concentration–response curves between different vessels. Values of p<0.05 were considered to be significant. In the text, average values are given with ±1 SEM.

**Drugs**

Drugs used and their sources were as follows: U46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin F2α, The Upjohn Co., Kalamazoo, Mich.), endothelin-1 (Auspep, Melbourne, Australia), 5-hydroxytryptamine creatinine sulfate (serotonin) and acetylcholine bromide (Sigma Chemical Co., St. Louis, Mo.), sodium nitroprusside (Nipride, Roche Products, Sydney, Australia), cromakalim (Beecham, Dandenong, Australia), arginine vasopressin (Auspep), UK14304 (5-bromo-6[2-imidazolizin-2-ylamino]-quinoxaline, Pfizer, Sandwich, Kent, UK), and methoxamine hydrochloride (a gift from Dr. Moncada, Wellcome Research Laboratories, Beckenham, Kent, UK).
TABLE 1. Passive Characteristics of Coronary Arteries Determined From the Normalization Routine in the Myograph

<table>
<thead>
<tr>
<th>Group 1</th>
<th>n</th>
<th>D1</th>
<th>P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal arteries</td>
<td>15</td>
<td>578.5±27.6*</td>
<td>62.1±3.5</td>
</tr>
<tr>
<td>Collateral arteries</td>
<td>15</td>
<td>510.7±43.6*</td>
<td>62.9±4.0*</td>
</tr>
<tr>
<td>Subgroup 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal arteries</td>
<td>4</td>
<td>615±34.8</td>
<td>57±2.9</td>
</tr>
<tr>
<td>Pseudonormal arteries</td>
<td>4</td>
<td>541.3±161.5*</td>
<td>57.5±2.9*</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Set a</td>
<td>4</td>
<td>470±39</td>
<td>58.5±5.5</td>
</tr>
<tr>
<td>Set b</td>
<td>4</td>
<td>512.8±50.8†</td>
<td>60.5±2.2†</td>
</tr>
</tbody>
</table>

Values are mean±1 SEM. n, Number of arteries (one per dog); D1, internal diameter when stretched on the myograph wires to 0.9 times internal diameter at an equivalent transmural pressure of 100 mm Hg; P1, transmural pressure calculated from P1=2T/D1, where T is the wall tension at D1 (see "Materials and Methods"); group 1, mongrel and greyhound dogs prepared with a casein occluder; subgroup 1, control (normal) and collateral arteries; subgroup 2, control (normal) arteries and arteries removed from small branches of the circumflex coronary artery distal to the occluder but branching away from the left anterior descending coronary artery (pseudonormal arteries); group 2, unoperated greyhound dogs; set a, normal vessels that were treated with low K+ before relaxation to acetylcholine and cromakalim and high K+ before sodium nitroprusside; set b, normal vessels that were treated with high K+ before acetylcholine and cromakalim and low K+ before sodium nitroprusside. *p=NS compared with corresponding value for normal arteries; †p=NS compared with corresponding value for set a.

Results

Pressure Gradient

In each of the five hearts measured, there was a significant (>5 mm Hg) pressure gradient between the LAD (120±8 mm Hg, n=5) and the CCA distal to the casein occluder (104±8 mm Hg). The average gradient (LAD–CCA) was 16±3 mm Hg (p<0.05), and the ratio of CCA to LAD pressures was 0.86±0.03.

Passive Vessel Characteristics

At normalization, the internal diameters at D1 (see "Materials and Methods") were not significantly different between strains for normal (one-way ANOVA, t=1.13) or collateral (t=0.12) arteries, allowing the data to be pooled for the mongrel and greyhound vessels. These combined data (n=15 vessels in each vessel type) showed no difference in D1 or P1, suggesting that the vessels were well matched in their passive characteristics. Similarly, the pseudonormal arteries had D1 and P1 values not significantly different from the normal arteries. In the four normal hearts (group 2), taken to test the functional antagonism of high versus low K+, the vessels in each treatment group were again well matched for size and passive distending pressure (Table 1).

As part of the normalization program, the values for tension (T) and diameter from each artery (D) were fitted to a linear equation:

\[ I_n(T) = I_0(\text{intercept}) + \text{slope } D \]

The average of these fitted slopes was 0.0092±0.0008 (mean±1 SEM) for 15 normal and 0.0112±0.001 for 15 collateral arteries (p=NS, unpaired t test), indicating that the passive compliance of the two groups of vessels was similar. Knowing the average diameter (Dw) at an equivalent transmural pressure of 100 mm Hg, the average tension was calculated and inserted in the equation above to determine the average intercept.

Membrane Potential

In the first cell impaled from each vessel that gave a satisfactory stable membrane potential reading for more than 2 minutes, the membrane potential was −44.1±1.3 mV in 11 normal arteries. The membrane potential of 14 collateral arteries tested was −55.2±1.5 mV, significantly more hyperpolarized than the control arteries (t=5.2, p<0.05).

Vasoconstrictor Agents

The majority of the concentration–response curves to the vasoconstrictor and dilator agents were performed on one collateral and one normal coronary artery segment each from seven mongrel dog hearts and from eight greyhound hearts. The contraction of the collateral arteries in response to a maximal depolarizing concentration of KCl (124 mM) was significantly less (61%) than in the normal arteries of similar size (Figures 2 and 3). This reduction in contraction in collateral arteries to the highest concentrations tested was also observed for endothelin (100 nM, only 33% of normal arteries) and U46619 (300 nM, 55% of normal arteries) (Figure 2). The collateral arteries tended to contract more strongly to arginine vasopressin than the normal arteries, but the maximum contraction in these collateral arteries was only 12% of the maximum contraction to K+ (Figure 3). The response to endothelin-1 (30 nM), U46619 (100 nM), and arginine vasopressin (10 nM) for the normal arteries was 64%, 30%, and 3% of the K+ contraction, respectively, and 33%, 26%, and 12% for the collateral vessels. To analyze the changes in sensitivity, as given by EC50, it is necessary to fit a logistic curve to each concentration–response curve. Full concentration–response curves were not obtained for endothelin-1 or U46619; therefore, accurate estimates of maximum contraction and EC50 could not be determined.

In the normal and collateral arteries from the mongrel dogs and the two greyhound dogs tested, neither serotonin (0.1–10 μM) nor methoxamine (0.1–10 μM) caused any contraction.

Vasodilator Agents

Arteries were precontracted to a steady level of force with K+ (25–40 mM). This submaximal K+ concentration caused a greater contraction (ΔP mm Hg) in normal arteries than in the collateral arteries (Figure 4). The paired experiments of one collateral and one normal artery from the same heart in each double myograph chamber ensured that the
same concentration of K⁺ was used. Relaxation responses to the vasodilator agents, expressed as a percentage of the K⁺ contraction, indicated that sodium nitroprusside (endothelium independent) had a significantly greater range of relaxation in the collateral arteries compared with the normal vessels; this was not the case for acetylcholine (endothelium dependent) and cromakalim, the K⁺ channel opener (Figure 4). The EC₅₀ values for the fitted acetylcholine curves in greyhound arteries were 6.6±0.1 (−log M, mean±1 SEM) in seven normal arteries and 6.6±0.2 in seven collateral arteries. For sodium nitroprusside, the EC₅₀ values from five mongrel dog hearts were 5.4±0.1 and 5.6±0.1 in normal and collateral vessels, respectively. Comparison of the absolute units of ΔP showed that the stronger contractions in the normal arteries to K⁺ may have led to the reduced relaxation in these arteries compared with the collateral vessels (Figure 4).

The other endothelium-dependent vasorelaxant tested, UK14304, was without effect in either normal or collateral vessels.

**High and Low K⁺ Concentration**

From a separate group of normal greyhound hearts (group 2), the relaxation response to acetylcholine, sodium nitroprusside, and cromakalim were assessed in the presence of high (40 mM) or low (25–30 mM) K⁺. K⁺ (40 mM) caused a greater contraction than 25–30 mM in all experiments, and the relaxation curves to all three agents were reduced in range (taken as a percentage of K⁺ contraction) without an apparent shift in sensitivity (Figure 5). For acetylcholine, the fitted EC₅₀ in low K⁺ was 6.7±0.4 (−log M) and in high K⁺ was 6.9±0.2 (−log M) (p=NS, unpaired t test).

**Pseudonormal Arteries**

From four greyhound hearts, two arteries were set up in the same myograph chamber, one from a small branch distal to the casein occluder but not directed toward the branches of the LAD and one normal coronary artery. The contraction responses (ΔP) to KSS were 290±33 mm Hg in normal arteries and 271±16 in pseudonormal vessels (n=4 in each group). The concentration–response curves for endothelin and U46619 were not significantly different between the two types of arteries (Figure 6). The relaxation curves to acetylcholine and cromakalim were also similar between the two types of vessels (Figure 7).

**Morphometry**

The morphology and morphometry measurements were done on segments of arteries that had been mounted in the myographs. Although from the same
heart and subjected to the same protocol, these vessels were not necessarily the same as those used to calculate the reactivity data since more than one vessel of each type was removed from each heart. Light microscopy and dissection revealed that collateral arteries had thick fibrous tissue on their adventitial surface in contrast to the normal arteries (and pseudonormal arteries, not shown) of similar size (Figure 8). In addition, the collateral arteries had a partially ruptured (incomplete) internal elastic lamina and much thickened neointima covered by endothelium (Figure 8). It was apparent from light microscopy that the wires used to mount the collateral arteries in the myograph had pushed through the neointima to the border of the media. This suggested that the diameter of the vessel measured during the normalization procedure would more closely correspond to the medial/neointimal border. The orientation of the neointimal smooth muscle cells was variable but predominantly longitudinal. The morphology of the pseudonormal arteries was similar to that of the normal coronary arteries.

In the fixed vessels, morphometric analysis of the cross-sectional areas of the adventitia, media, neointima, and lumen showed that there was no significant difference (by split plot analysis of variance, F₁, 55, t = 0.19) between these areas for the six mongrel collateral arteries and the eight greyhound collateral arteries, allowing us to pool these data for the two strains. For the normal arteries, the three areas measured were adventitia, media, and lumen. There was a significant difference between greyhound and mongrel vessels (F₁, 41, t = 2.9) principally because the medial area was significantly greater in the greyhound normal arteries (p < 0.05, unpaired t test). This is consistent with the greyhound arteries being slightly greater in diameter when passively stretched in the myograph (D₃₁ = 613 ± 45.8 μm, n = 8) than normal mongrel arteries (D₃₁ = 539 ± 23 μm, n = 7). The measurement of the areas showed significantly reduced lumen and media and increased adventitia.

Figure 4. Average concentration–relaxation curves for acetylcholine (Ach), sodium nitroprusside (SNP), and cromakalim (Crom) in collateral (C) and normal (N) coronary arteries preconstricted to a steady submaximal level of force by K⁺ (25–35 mM). Relaxation is presented in absolute scale (ΔP mm Hg) (top panels) or as percent relaxation of the initial K⁺ contraction (bottom panels). Error bars at selected points are ± 1 SEM from 10–13 arteries (one per dog) each in the N and C groups for Ach and Crom but from six arteries (greyhounds only) for Crom.

Figure 5. Average concentration–relaxation curves for acetylcholine (−log M) in eight normal coronary arteries taken from four greyhound dogs without casein occluders (group 2). These vessels were preconstricted to a higher (n = 4) or lower (n = 4) level of equivalent transmural pressure (ΔP mm Hg) by exposure to high KCl (40 mM) or low KCl (25–30 mM). Top panel: Relaxation expressed in absolute levels of ΔP (mm Hg). Bottom panel: Response expressed as percent relaxation of precontracted force. Error bars at selected points are ± 1 SEM.

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in collateral arteries compared with the normal vessels (Figure 9).

From the pooled data for greyhound and mongrel vessels, morphometric area analysis was used to derive the various radii. The radii measured to the adventitial/medial border were not significantly different among the normal, collateral, or pseudonormal vessels (Table 2, Figure 9). The collateral arteries had a thickened adventitia (Table 2, Figure 9) but a medial thickness less than half that of the normal arteries ($p<0.05$). The medial thickness/luminal radius ratio was not significantly different between mongrel and greyhound normal arteries ($t=0.51$, df=12) or collateral arteries ($t=0.88$, df=12). In the collateral arteries, the luminal radius was only 66% of that in the normal vessel.

The pseudonormal arteries were similar to the normal coronaries except that the media tended to be thinner, giving rise to a reduced medial thickness/luminal radius ratio (Table 2). The area of adventitia was significantly less in these arteries compared with normal arteries (Table 2).

Theoretical calculations were made to translate the data from the isometric tension measurements in the myograph to blood vessels under isotonic conditions (see “Appendix”). Here the transmural pressure would theoretically be allowed to remain constant and the diameter would decrease as the smooth muscle contracted in response to the vasoconstrictor stimuli (Figure 10). Taking the starting luminal area as only 25% in the collateral arteries, the theoretical data showed that, even though the collateral arteries contracted poorly (isometrically) to endothelin-1 and U46619, these vessels would still contract sufficiently to have a zero lumen at concentrations $\sim 10^{-7}$ M U46619 and $<3 \times 10^{-8}$ M endothelin-1 (Figure 10). Concentrations even 10-fold higher than these would not have occluded the normal arteries (Figure 10) (see “Appendix”).

**Discussion**

This study shows that epicardial collateral coronary arteries with an internal diameter of $\sim 500 \mu m$ studied with a myograph contract poorly in response to endothelin-1, the thromboxane A$_2$ mimetic U46619, and the non–receptor-mediated constrictor agent KCl compared with normal coronary arteries of similar diameter. Relaxation, on the other hand, was apparently unaltered in collateral arteries. Acetylcholine, sodium nitroprusside, and cromakalim were as effective in collateral arteries as in normal vessels. Thus, on this evidence it would appear that these mature epicardial collateral vessels are, if anything, “protected” against vasospasm, with contractility reduced but relaxation maintained.

However, these studies can only provide information on the reactivity of the artery with regard to the contractile range of the vessels as determined from isometric recordings in vitro with a myograph. On the other hand, the morphological data taken together
FIGURE 8. Light micrographs of fixed cross sections of normal (N, top panel) and collateral (C, bottom panel) coronary arteries after removal from the myograph. Note the thickened adventitia (a), thin media (m), and neointimal (ni) thickening in the collateral artery. The indentation in the intima was caused by the supporting wire in the myograph. Detail of the neointimal smooth muscle cells and ruptured internal elastic lamina (iel) in the collateral artery can also be seen. Bar, 50 μm.
with the myographic information bring a greater understanding of how these vessels may behave in vivo, where the vessel will change in luminal diameter and alter blood flow. Our theoretical calculations outlined in the "Appendix" show that these vessels are left in a precarious position if subjected to vasoconstrictor stimuli. Even allowing for the reduced contractile performance due to a paucity of the media, our prediction is that the collateral arteries are capable of reducing flow to zero principally because of their smaller lumen due to the neointimal thickening when compared with normal coronary arteries of similar internal diameter to the intimal/medial border.

Selection of Collateral Vessels and Morphology

We chose to compare the isometric contractility of epicardial collateral arteries with similarly sized (at
least in diameter to the intimal/medial border) normal coronary arteries. These collateral vessels probably arose from the initial event of a ruptured internal elastic lamina when the thin-walled native collateral arteries were subjected to a marked rise in transmural pressure and flow as the casein occluder closed the CCA. Even more than 6 months later, in a subgroup of dogs, we measured a pressure gradient of 16±3 mm Hg between the CCA distal to the occluder and the LAD, which is comparable with a gradient of 21±3 mm Hg recently published in similar experiments in mongrel dogs. Not all blood vessels distal to the casein occluder became collateral vessels to communicate with the branching vessels from the LAD. Indeed, our pseudonormal arteries did not show the characteristic neointimal thickening or ruptured internal elastic lamina but only a slightly thinner media, perhaps as a result of the reduced transmural pressure.

In the collateral arteries, the neointima is probably the result of the early rupture of the internal elastic lamina and invasion of serum mitogens, with the resulting longitudinally orientated smooth muscle cells surrounded by collagen fibers and elastin. These smooth muscle cells are probably of a noncontractile phenotype and, once they are orientated longitudinally, contribute little to circumferential contraction.

Normalization

All arteries were subjected to an individual diameter–wall tension procedure to allow a resting wall tension to be chosen that was appropriate for the passive characteristics of the vessel. Histological evidence showed that the mounting wire (40-μm diameter) probably broke through the neointimal layers in the collateral arteries and stretched the wall at the medial/internal elastic laminal border as it did for the normal and pseudonormal arteries. Therefore, the three types of arteries had similar internal diameters, presumably measured at the intimal/medial border (Table 1). We consider that using a passive length–tension curve to set initial wall tension is preferable to using an agent to generate active force to find the optimal passive stretch for maximum contraction. This latter technique assumes that the agonist action is unaltered between normal and test arteries—an assumption that may not be correct.

Evidence that the three types of arteries were subjected to similar passive tensions was the calculated transmural pressures (P=T/r), with the means ranging over only 6 mm Hg for the six groups studied (Table 1).

Contraction

The maximum contraction to K\(^+\) depolarization was reduced in the collateral arteries. This finding was also observed for the thromboxane A\(_2\) mimetic U46619 and endothelin-1 without apparent change in threshold sensitivity. Thus, the defect is probably at the level of the contractile machinery and not at the membrane receptors or second message transduction. Support for this conclusion is that the medial thickness of the collateral arteries was only 47% of the normal arteries (Table 2). This assumes that the thick neointima contributes little to the isometric contraction on the grounds that the neointimal smooth muscle cells are of the synthetic (noncontractile) phenotype and predominantly longitudinally oriented. The membrane was significantly more hyperpolarized in the collateral vessels than in the normal vessels, which could also reduce the response to constrictor agents. On the other hand, the result with K\(^+\) (124 mM) argues against the membrane potential hypothesis, since the depolarization would be complete with K\(^+\) at 124 mM, and it should have then generated a similar level of force in both types of vessels.

Vasopressin at 10 nM contracted the normal coronary vessels to only 3% of the K\(^+\) (124 mM) response but to 12% in the collateral arteries. Although the response to vasopressin seems to be increased in collateral arteries (opposite the response to endothelin-1, U46619, or K\(^+\)), it was not as great as that reported in similar-sized collateral arteries where vasopressin (300 nM) caused 90% of the contraction to K\(^+\) (100 mM). We did not use concentrations >30 nM in our study, because there was no obvious concentration–response curve developing above 30 nM.

Vasopressin (1 millilunit/ml) was more effective in coronary collateral microvessels (<200-μm diameter) than in normal microvessels. Thus, although there is some variability in the response to vasopressin in collateral arteries, they do appear to be more reactive than normal coronary arteries matched for size—a trend not shared by other vasoconstrictor stimuli. We could not test the role of α\(_1\)-adrenoceptors and serotonin receptors in these arteries, since neither methoxamine nor 5-hydroxytryptamine caused any contraction in the normal or collateral arteries.

Relaxation

In isolated blood vessels in vitro, there is usually very little resting active force from which to examine relaxation. Therefore, the dilemma is to choose an appropriate level of precontraction by applying a concentration of a constrictor that gives a submaximal level of sustained force. In these experiments, K\(^+\) was the constrictor agent chosen, but as discussed earlier it was less active in the collateral vessels than in the control vessels. In terms of relaxation, the two parameters of interest are the sensitivity and the range of the relaxation–response curve. Both the range of relaxation and/or sensitivity to a dilator agent can be markedly altered by the level of contractile force generated. If K\(^+\) causes contraction by membrane depolarization and Ca\(^{2+}\) entry, then the force generated is functionally antagonized by endothelin-derived relaxing factor generation (via acetylcholine) and cGMP formation, leading to Ca\(^{2+}\) sequestration. We found that the normalized range (the percentage) of relaxation was greater in collat-
eral arteries than in normal arteries in response to sodium nitroprusside and that the trend was similar for acetylcholine and cromakalim (Figure 4). Although high K+ concentrations do attenuate cromakalim-induced relaxation in the canine coronary artery,\textsuperscript{20} we used a low concentration range of K+ that was the same for both normal and collateral arteries to avoid this potential problem.

One conclusion from these findings is that the dilators have higher efficacy in collateral vessels than in the normal arteries and that the endothelium-derived relaxing factor stimulant acetylcholine is as potent a dilator as sodium nitroprusside. However, we examined the implication of the lower level of contractile response on the subsequent response to dilators in a subset of experiments using arteries from normal greyhounds. At the lower concentration of K+ (25–30 mM), all three vasodilators relaxed normal vessels over a greater range than those exposed to high K+ (40 mM). In light of this finding, we suggest that there is no evidence that endothelium-dependent relaxation is impaired in the collateral arteries. This is in contrast to a recent report in which the relaxation to acetylcholine and ADP (but not to the ionophore A23187) was reduced in collateral microvessels (100–200 μm) compared with normal microvessels.\textsuperscript{8} It is possible that the microvessels in this latter study\textsuperscript{8} were new vessels formed after CCA occlusion rather than the expanded native collateral vessels that were probably the source of vessels that we studied. In support of such an interpretation is the apparent lack of neointimal thickening in the microvessels.\textsuperscript{8} Our result of apparently normal response to acetylcholine is of considerable interest, given the generally concentric neointimal thickening in the collateral vessels. We have previously shown that endothelium-dependent relaxation to cholinomimetics is apparently normal in the presence of neointimal thickening in rabbit carotid arteries.\textsuperscript{19}

The lack of relaxation in response to UK14304 in either normal or collateral coronary arteries suggests that the α1-adrenoceptor is probably lacking on the endothelial cells in either type of vessel in the dog. This confirms the finding that norepinephrine did not relax the greyhound coronary microvessel (467 μm i.d.) in contrast to the pig microvessel;\textsuperscript{21} on the other hand, both norepinephrine and UK14304 relaxed canine large coronary arteries in an endothelium-dependent manner.\textsuperscript{14}

In summary, we found that surface mature collateral coronary arteries from dog hearts contract poorly, probably because they have a thinner media but relax normally in response to endothelium-dependent and to endothelium-independent vasodilators. The neointimal thickening and subsequent reduction in free luminal area presumably compromise the vessels in terms of blood flow capacity, especially during exercise\textsuperscript{22,23} or in the presence of an active vasoconstrictor such as endothelin or thromboxane. These studies add experimental evidence to the premise that collateral vessels are not merely passive conduits and should be considered as possible sites for the generation of vasospastic angina or unstable angina.\textsuperscript{24}

Appendix

The traditional method of measuring blood vessel reactivity in vitro is to apply a passive radial force (stretch) to a ring segment or spiral-cut strip suspended in an organ bath. Ideally, the amount of passive force is chosen to be optimal for the maximum development of active force when the smooth muscle is stimulated by a constrictor agent. Both the passive force and active force are measured isometrically (i.e., where the smooth muscle shortens only to a minimal degree). However, in the intact vasculature the more general case is where perfusion pressure remains fairly constant but flow decreases as the luminal area falls because of the shortening of the circumferentially arranged medial smooth muscle.

In the present work, the collateral arteries have two distinct features: 1) a reduced isometric contractile response to three vasoconstrictors that is probably due to a reduced medial smooth muscle content and 2) a significant neointimal thickening that compromises the available luminal area. Here theoretical calculations have been made to predict how these collateral vessels might behave if the contractile and morphometric properties are considered together in an environment where vasoconstriction causes the circumference to shorten (isotonic) with the reduction of luminal area already compromised by the neointima.

Consider the maximally dilated vessel as a thin-walled sphere being distended under a passive (p) transmural pressure (Pp). From Laplace

$$T_p = r_p \cdot P_p$$  \hspace{1cm} (1)

where Tp is the circumferential passive wall tension and rp is the radius.

Under isometric conditions, a vasoconstrictor stimulus causes a rise in active (a) tension (Ta) where the radius (ra) remains constant. Under isotonic conditions, a vasoconstrictor stimulus causes a fall in radius (ra), but the wall tension (T) remains constant (T = T).

For isometric conditions, ra = ra. The equation for activation is

$$T_a = r_a \cdot P_a$$  \hspace{1cm} (2)

Since Tp = Tp/Pp from Equation 1, combining Equations 1 and 2 yields

$$T_a = T_p \cdot P_a / P_p$$

For isotonic conditions, T = T. The equation for activation is

$$T_a = r_a \cdot P_a$$  \hspace{1cm} (3)

Since Tp = rp \cdot Pp from Equation 1, combining Equations 1 and 3 yields ra \cdot Pa = rp \cdot Pp or

$$ra = rp \cdot P_p / P_a$$
From the isometric experimental data, $P_s$ was calculated from the relation $T/R_{pa}$ for each concentration of endothelin-1 or U46619. Since the passive radius ($r_0$), passive transmural pressure ($P_p$), and $P_s$ were known, it was possible to calculate the active radius ($r_a = r_0 \cdot P_p/P_s$) under theoretical isometric conditions. These calculations are based on the internal radius to the intimal/media boundary in the normal vessels. But because the mounting wires broke through the neointima in the collateral arteries, the measurements of $r_n$ can only relate to the neointimal/media border in these arteries. Before the free luminal area can be calculated ($\pi r_a^2$) for the collateral arteries, the area of neointima must be calculated from the average morphology results.

Results showed that the luminal area in the absence of U46619 or endothelin-1 was 0.263 mm² in normal and 0.205 mm² in collateral arteries when internal diameter was taken from the myographic normalization (normal arteries, 579 µm; collateral arteries, 511 µm; Table 1). The neointimal area calculated from morphometry was 0.091 mm², reducing the free luminal area of the collateral arteries in the absence of constrictor tone to 55.7%. The resting free luminal area was thus 0.263 mm² in normal and 0.114 mm² in collateral arteries (Figure 10). Addition of vasodilator tone from endothelin-1 or U46619 reduced the free luminal area to zero in collateral arteries but not in normal arteries, indicating, at least theoretically, that the collaterals appeared more susceptible to spasm (zero flow) than the normal coronary arteries (Figure 10).

Theoretical calculations assume 1) that the measurement of the diameter at normalization in collateral arteries is to the neointimal/media border, a pattern that was observed in 13 of 14 collateral arteries at light microscopy, and 2) that the area of neointima is constant from the myographic experiment through the histological fixation process without distension. This latter assumption has been validated at least for the medial area of rat mesenteric artery, where changes in tension did not alter the estimations.25

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