Altered reactivity of coronary arteries located distal to a chronic coronary occlusion

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Rapps, Julie A., Michael Sturek, Allan W. Jones, and Janet L. Parker. Altered reactivity of coronary arteries located distal to a chronic coronary occlusion. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H1879–H1887, 1997.—The coronary vasculature located distal to a chronic occlusion (collateral-dependent) has been shown to exhibit altered reactivity to vasoactive agonists. Thus we evaluated effects of chronic coronary artery occlusion on vasmotor responsiveness of collateral-dependent arteries isolated from a canine model of Ameroid occlusion of the left circumflex (LCX) coronary artery. We compared in vitro responses of large (~1.3- to 1.4-mm-ID) and small (~0.6-mm-ID) LCX arteries located distal to an occlusion with responses of similar-sized segments of the unoccluded left anterior descending (LAD) coronary artery. α1-Adrenergic receptor-mediated contractile responses to norepinephrine (10−9−10−4 M) and phenylephrine (10−9−10−4 M) in the presence of propranolol were markedly enhanced in large LCX arteries compared with LAD arteries (P < 0.001). Prazosin (1 µM), an α1-adrenergic receptor antagonist, abolished contractile responses of LCX and LAD arteries to norepinephrine. Inhibition of nitric oxide synthesis with Nω-nitro-L-arginine methyl ester (100 µM) enhanced norepinephrine-induced contractions of LAD arteries to a greater extent than contractions of LCX arteries. We simultaneously measured myoplasmic free Ca2+ (fura 2 fluorescence ratio) and contractile responses in LCX and LAD arteries denuded of endothelium; norepinephrine-induced increases in myoplasmic free Ca2+ and contractile tension were significantly enhanced in LCX arteries compared with LAD arteries. In addition, large and small LCX arteries exhibited impaired relaxation in response to adenosine (10−8−10−3 M) compared with LAD arteries (P < 0.05). In contrast, relaxation in response to the β-adrenergic agonist isoproterenol (10−9−10−4 M) and sodium nitroprusside (10−10−10−4 M) was not significantly different in LCX and LAD arteries. Thus collateral-dependent coronary arteries exhibit enhanced α-adrenergic vasoconstriction and impaired vasorelaxation in response to adenosine. The enhanced α-adrenergic contractile responsiveness involves at least two mechanisms: 1) enhanced α1-adrenergic reactivity of smooth muscle and 2) decreased α-adrenergic-induced synthesis of nitric oxide by the endothelium.

MATERIALS AND METHODS

Induction of Chronic Coronary Artery Occlusion

Adult mongrel male dogs (25–35 kg) were anesthetized with acepromazine maleate (0.8 mg/kg sc) and pentobarbital sodium (25 mg/kg iv) and ventilated mechanically. An Ameroid constrictor (2.75–4.0 mm ID; Research Instruments and Manufacturing, Corvallis, OR), chosen to fit snugly around the vessel, was placed around the proximal LCX using sterile techniques. During surgery and recovery, dogs received buprenorphine hydrochloride (0.3 mg iv or im) as needed for...
pain relief. Antibiotics were given immediately before surgery (900,000 U penicillin im) and for 5 days after surgery (800 mg sulfamethoxazole and 160 mg trimethoprim). All experimental procedures were in accordance with the "Position of the American Heart Association on Research Animal Use" adopted on 11 November 1984 and were approved by the Animal Care and Use Committee of the University of Missouri.

Preparation of Coronary Artery Rings for Studies of Vasomotor Function

We studied vasomotor function of coronary arteries isolated from hearts 4 mo (123 ± 1 days) after implantation of the Ameroid occluder. Occlusion of the LCX artery was confirmed by visual inspection of the occluder on the day of each experiment. Complete occlusion of the LCX artery occurred in 68 of 75 dogs that were instrumented with an Ameroid occluder. We performed separate analyses on data collected from the subset of dogs in which sterile surgery was performed, but the Ameroid occluder did not produce occlusion.

On the day of the experiment, dogs were anesthetized with pentobarbital sodium (40 mg/kg), and the hearts were removed rapidly and placed in cold Krebs bicarbonate buffer. Hearts were kept in aerated iced Krebs buffer during isolation of coronary vessels. We isolated proximal portions of the LAD (normal) and LCX (collateral-dependent: distal to the Ameroid occluder) coronary arteries. These arteries had average inner diameters of 1.3–1.4 mm and were termed small conduit arteries. A matching epicardial branch of the LAD (normal) and LCX (collateral-dependent arteries) were studied in all arteries; relaxation responses to Ca2+ ionophore A-23187 (10 µM) and Bradykinin (1 µM) were not significantly different in LCX and LAD vessels, indicating preservation of endothelial integrity in collateral-dependent arteries.

Simultaneous Measurement of Fura 2 Fluorescence and Contractile Tension

Contractile tension and Ca2+ were measured simultaneously in large conduit coronary rings using a specially designed myograph and microfluorometry instrumentation and methods described previously (6, 32). Arterial rings (1-mm axial length) were carefully denuded of endothelium by gentle rubbing of the luminal surface with suture. Because the adventitia of these arteries produces high autofluorescence, a portion (~0.5 mm²) of the adventitia was removed. This carefully cleaned portion of the arterial ring was positioned directly over the objective during the experiment so that fura 2 fluorescence from the smooth muscle could be measured directly without the high autofluorescence of the adventitia. Smooth muscle of the arterial rings was loaded with fura 2 by incubation of the arteries with 10 µM fura 2 acetoxymethyl ester (fura 2-AM) for 2 h at 37°C. The fura 2-loading solution contained 0.5% cremophor el and 5% bovine serum albumin and was vortexed and sonicated for 1 min to increase solubilization of fura 2-AM. Arteries were rinsed for 30 min at 37°C to remove extracellular fura 2-AM.

Coronary rings were mounted on two stainless steel wires: one was attached to a digital micrometer to permit control of circumferential length (stretch) of the vessel, and the other was attached to a force transducer (Kulite Semiconductor Products, Leonia, NJ) for measurement of force. After being mounted on the myograph, arteries were lowered into a superfusion chamber positioned on the heated stage of a microfluorometry system described previously (32, 33). Light from a xenon arc lamp was passed to arterial rings via a liquid light guide through a rotating wheel containing 340- and 380-nm interference filters. The fluorescence emission at 510 nm was reflected to a photomultiplier tube with a dichroic mirror. The fluorescence was analyzed with an analog fluorometer signal processor and an analog-to-digital converter. Fluorescence and tension values were sampled every 5 s.

Arteries were superfused with Krebs buffer bubbled with 95% O2-5% CO2 and heated to 37°C. Each ring was stretched to Lmax (optimal length), as determined by repeated exposures to 80 mM K+ at increasing vessel lengths, as described above. Autofluorescence of arterial rings was determined at the end of each experiment by quenching the Ca2+-sensitive fura 2 fluorescence via exposure of the arteries to 10 mM MnCl2 and 5 µM ionomycin (6, 33). Fluorescence ratio was calculated after subtraction of vessel autofluorescence. Data acquisition and transformations were performed using AxoBASIC software customized for multichannel data acquisition (32, 33).

Solutions and Drugs

The Krebs bicarbonate solution for all experiments contained (in mM) 131.5 NaCl, 5.0 KCl, 1.2 NaH2PO4, 1.2 MgCl2, 2.5 CaCl2, 25 NaHCO3, and 10.1 glucose (bubbled with 95% O2-5% CO2, pH 7.4). This solution also contained 25 µM EDTA. For studies of α-adrenergic contractile responsiveness, 3 µM propranolol was added to the solution. Solutions were prepared using AxoBASIC software customized for multichannel data acquisition (32, 33).
with elevated $K^+$ concentrations used for depolarizing smooth muscle were produced by equimolar replacement of NaCl with KCl. Drugs were obtained from Sigma Chemical (St. Louis, MO) unless otherwise indicated. We purchased endothelin-1 from Peninsula Laboratories (Belmont, CA), ionomycin from Calbiochem (La Jolla, CA), PGF$_{2\alpha}$ from Upjohn (Kalamazoo, MI), and fura 2-AM from Molecular Probes (Eugene, OR).

Data Analyses

Contractile responses of coronary arteries were expressed as absolute values in grams of developed tension (force). The concentration of agonist causing 50% of the maximal response was designated EC$_{50}$ and was calculated using nonlinear regression analysis of the concentration-response data for each artery. Measurements of $C_{am}$ were expressed as the ratio of fura 2 fluorescence at 340- and 380-nm excitation wavelengths because of uncertainties regarding extrapolation of in vitro calibrations to in vivo measurements (33). Concentration-response curves were compared using two-way analysis of variance for repeated measures. Subsequently, differences between individual points were ascertained using Fisher’s test for least significant difference. We determined that data from experiments in which we simultaneously measured fura 2 fluorescence and contractile tension had non-Gaussian characteristics that could lead to erroneous conclusions if normal theory-dependent analyses were used. Therefore, these data were analyzed using Wilcoxon signed-ranks test. For all analyses, $P < 0.05$ was considered significant. Values are means ± SE.

RESULTS

Artery Dimensions and Passive Characteristics

Dimensions of the arterial rings used in this study are presented in Table 1. Proximal large conduit arteries had mean inner diameters averaging 1.3–1.4 mm. Large conduit LCX arteries tended to be slightly smaller than corresponding LAD arteries. Inner diameters of small conduit arteries averaged ~0.6 mm. Length-active tension relationships were qualitatively similar in large and small conduit LAD and LCX arteries. $L_{max}$ and resting tension at $L_{max}$ were not significantly different between the two groups of arteries (data not shown).

Contractile Responses

Small conduit arteries. We evaluated contractile responses of small conduit coronary arteries to $K^+$, PGF$_{2\alpha}$, endothelin, and norepinephrine. Contractions of small LCX and LAD arteries in response to increasing concen-

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Values are means ± SE in mm; n, total number of rings studied in each group. LAD, left anterior descending; LCX, left circumflex.

iments of $K^+$ (5–100 mM), PGF$_{2\alpha}$ (10$^{-8}$–10$^{-4}$ M), and endothelin (10$^{-10}$–10$^{-8}$ M) were not significantly different (P > 0.05, data not shown). Norepinephrine did not produce significant increases in contractile tension in small LCX or LAD arteries. We did not observe any alteration in the contractile responsiveness of small arteries located distal to a chronic coronary occlusion.

Large conduit arteries. Contractile responses of large conduit arteries to $K^+$ and PGF$_{2\alpha}$ are presented in Fig. 1. Concentration-dependent contractile responses of large LAD and LCX arteries to depolarization with $K^+$ and PGF$_{2\alpha}$ were not significantly different. Contractile responses to endothelin (30 nM) were also not significantly different between large LCX and LAD arteries (data not shown).

In contrast to responses to $K^+$ and other receptor-dependent vasoconstrictors, concentration-dependent contractions of large LCX arteries to the $\alpha_1$- and $\alpha_2$-adrenergic agonist norepinephrine were markedly enhanced compared with contractions of similar-sized LAD arteries (P < 0.001; Fig. 2A). Norepinephrine-induced contractions of LAD and LCX arteries were abolished in the presence of the $\alpha_1$-adrenergic receptor antagonist prazosin (1 $\mu$M). Contractions of large LCX arteries in response to the selective $\alpha_1$-adrenergic receptor agonist phenylephrine were also significantly larger than contractions of LAD arteries (P < 0.001; Fig. 3). Importantly, norepinephrine concentration-response curves of LCX arteries isolated from the subset of animals in which the Ameroid did not produce coronary occlusion were not significantly different from those of LAD arteries isolated from the same dogs (Fig. 2B).

To determine the role of synthesis of nitric oxide in modulating $\alpha$-adrenergic vasoconstriction, we evaluated norepinephrine-induced contractions in the presence of nitric oxide synthase inhibitor L-NAME (100 $\mu$M). Concentration-dependent increases in contractile tension were significantly enhanced in large LAD arter-
ies pretreated with L-NAME compared with paired LAD arteries not treated with the inhibitor (P < 0.001; Fig. 2 vs. Fig. 4). In contrast, pretreatment with L-NAME did not significantly alter the concentration-response relationship to norepinephrine in large LCX arteries (P > 0.05). In the presence of L-NAME, contractile responses of LCX arteries to norepinephrine remained significantly enhanced compared with contractions of LAD arteries (P < 0.01; Fig. 4).

In contrast to differential effects of L-NAME on norepinephrine-induced contractions of LCX and LAD arteries, the relative responsiveness of LCX and LAD arteries in response to K+ and PGF2α was not altered by the presence of L-NAME; contractions of LCX and LAD arteries remained not different after pretreatment with L-NAME (data not shown). Thus a disparity in the effects of inhibition of nitric oxide synthesis on contractile responses of LCX and LAD arteries was observed only with norepinephrine.

Ca2+ Responses to Norepinephrine

In additional studies we simultaneously measured Ca2+ and contractile responses to norepinephrine in large conduit LCX and LAD arteries to determine whether the enhanced α1-adrenergic contractile responsiveness of LCX arteries involved concomitant alterations in Ca2+ regulation. Simultaneous measurements of fura 2 fluorescence ratio and contractile tension were performed in large arteries denuded of endothelium and, thus, represented responses of the smooth muscle that were independent of effects of vasoactive substances released from the endothelium. Figure 5 illustrates average time-dependent increases in Ca2+ (ratio) and contractile tension in response to norepinephrine (3 and 30 µM) in large LCX and LAD arteries. Norepinephrine-induced increases in Ca2+ (30 µM) and contractile tension (3 and 30 µM) were significantly enhanced
in smooth muscle of LCX arteries compared with LAD arteries (P < 0.05).

Relaxation Responses

Adenosine. We evaluated concentration-dependent relaxation in response to adenosine in large and small conduit arteries preconstricted with PGF$_{2\alpha}$ (30 µM) or K$^+$ (30 mM). Adenosine-induced relaxation of large conduit LCX arteries was significantly impaired compared with relaxation of LAD arteries (Fig. 6). Relaxation to adenosine was also significantly attenuated in small conduit LCX arteries compared with similar-sized LAD arteries, but only when these arteries were preconstricted with PGF$_{2\alpha}$ (data not shown).

In contrast to results obtained from occluded hearts, adenosine-mediated relaxation was similar in large and small LCX and LAD arteries isolated from the subset of dogs without occlusion of the LCX artery (data not shown). These arteries were preconstricted with PGF$_{2\alpha}$. Maximal relaxation of large LCX and LAD arteries from nonoccluded dogs averaged 65 ± 9 and 78 ± 7, respectively (P > 0.05). Maximal relaxation of small LCX and LAD arteries averaged 85 ± 9 and 83 ± 2, respectively (P > 0.05).

Effects of inhibition of nitric oxide synthesis were evaluated on adenosine-mediated relaxation of large conduit arteries preconstricted with PGF$_{2\alpha}$. Pretreatment with L-NAME did not inhibit adenosine-induced relaxation of LCX or LAD arteries. Adenosine relaxation of large conduit LCX arteries remained significantly impaired in the presence of L-NAME (P < 0.01, data not shown).

Isoproterenol. Relaxation in response to the β-adrenergic agonist isoproterenol was evaluated in large and small conduit coronary arteries. In contrast to relaxation responses to adenosine, isoproterenol-induced relaxation of large and small conduit LCX arteries was not significantly different from relaxation of similar-sized LAD arteries (P > 0.05, data not shown).

Nitroprusside. Relaxation in response to sodium nitroprusside was evaluated in large and small conduit coronary arteries. Relaxation in response to nitroprusside was not significantly different between large (Fig. 7) and small (data not shown) LCX and LAD arteries.

DISCUSSION

The current study documents important alterations in the vasomotor responsiveness of coronary vasculature located distal to a chronic occlusion. We determined that large epicardial arteries located distal to the occlusion exhibit enhanced contractile responsiveness to the α-adrenergic agonists norepinephrine and phenylephrine in the presence of β-adrenergic receptor blockade. In contrast to enhanced α-adrenergic-mediated vasoconstriction, contractions of LCX arteries in response to K$^+$, PGF$_{2\alpha}$, and endothelin were not altered. Another new finding is that relaxation of large and small collateral-dependent arteries in response to adenosine is impaired. Importantly, α-adrenergic-mediated vasoconstriction and adenosine-mediated vasorelaxation were not altered in LCX arteries isolated from dogs in which the ameroid did not produce coronary occlusion. These findings indicate that these
Heart changes appear to result from the surgical procedures. Thus chronic coronary occlusion produces potentially detrimental changes in vasomotor reactivity of the distal vasculature, resulting in an imbalance between vasoconstriction (enhanced \(\alpha_1\)-adrenergic vasoconstriction) and vasodilation (impaired adenosine-mediated vasodilation). Pathophysiological consequences of this imbalance may involve increased vasoconstric
tor activation. However, Cam and contractile responses to \(\alpha_1\)-adrenergic receptor stimulation, since enhanced contractility is involved in the enhanced contractile responsiveness of LCX arteries to norepinephrine and that this impaired nitric oxide production is likely selective for \(\alpha_1\)-adrenergic stimulation.

Results from experiments in which we simultaneously measured \(C_{am}\) and contractile responses to norepinephrine in coronary arteries denuded of endothelium indicate that the enhanced \(\alpha_1\)-adrenergic contractile responsiveness also involves changes in reactivity of smooth muscle of LCX arteries independent of alterations in endothelial function. Norepinephrine elicited minimal \(C_{am}\) and contractile responses in LAD arteries, consistent with the report of Shogakiuchi et al. (31) that normal coronary artery smooth muscle cells do not exhibit significant \(C_{am}\) responses to \(\alpha_1\)-adrenergic receptor activation. However, \(C_{am}\) and contractile responses to norepinephrine were enhanced in parallel in smooth muscle of LCX arteries located distal to the chronic coronary occlusion. \(C_{am}\) and contractile force are not necessarily directly related during agonist stimulation, even in normal vascular smooth muscle. Under many circumstances, contractions can be sustained with little or no increase in \(C_{am}\) (25). However, our simultaneous measurements of \(C_{am}\) and contraction indicate that the augmented \(\alpha_1\)-adrenergic contractions of LCX arteries do result, at least in part, from parallel enhancement of \(\alpha_1\)-adrenergic-mediated increases of \(C_{am}\). Furthermore, our results suggest that enhanced contractile and \(C_{am}\) responses observed in LCX arteries are selective for \(\alpha_1\)-adrenergic receptor stimulation, since enhanced responses were not observed to \(K^+\), PGF\(_{2\alpha}\), or endothelin (Fig. 1, other data not shown). Therefore, our results indicate that \(\alpha_1\)-adrenergic receptors and/or signaling

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**Fig. 7. Nitroprusside-induced relaxation of large (A) and small conduit (B) coronary arteries preconstricted with 30 mM K\(^+\). Relaxation of collateral-dependent LCX and normal LAD arteries in response to nitroprusside was identical. Values are means \pm SE of number of animals in parentheses.**

Alterations do not reflect regional differences between normal LCX and LAD coronary arteries, nor do these changes appear to result from the surgical procedures. Thus chronic coronary occlusion produces potentially detrimental changes in vasomotor reactivity of the distal vasculature, resulting in an imbalance between vasoconstriction (enhanced \(\alpha_1\)-adrenergic vasoconstriction) and vasodilation (impaired adenosine-mediated vasodilation). Pathophysiological consequences of this imbalance may involve increased vasoconstrictor tone and/or an increased propensity for coronary vasospasm, potentially resulting in initiation or aggravation of myocardial ischemia in the collateral-dependent region of hearts with chronic coronary occlusion.

**Mechanisms of Enhanced \(\alpha_1\)-Adrenergic Contractile Responsiveness**

Adrenergic stimulation of normal canine coronary arteries elicits multiple effects. In large coronary arteries, vasodilation mediated by \(\beta\)-adrenergic receptors is opposed by \(\alpha_2\)-adrenergic receptor-induced vasoconstriction (4, 10). Although activation of \(\alpha_2\)-adrenergic receptors produces vasoconstriction of canine coronary arteries (9, 35), \(\alpha_2\)-adrenergic receptors do not normally mediate adrenergic vasoconstriction of large coronary arteries (4). Instead, \(\alpha_2\)-adrenergic receptor stimulation has been demonstrated to produce endothelium-dependent relaxation in large canine coronary arteries (1, 5). \(\alpha_2\)-Adrenergic-mediated relaxation of canine coronary arteries is attenuated by inhibitors of nitric oxide synthesis (5), indicating that \(\alpha_2\)-adrenergic receptors on the endothelium of canine coronary arteries are coupled to the synthesis/release of nitric oxide.

To gain insight into which subtype of \(\alpha_1\)-adrenergic receptor is involved in the altered contractile responsiveness of LCX arteries, we evaluated effects of the \(\alpha_1\)-adrenergic receptor antagonist prazosin on norepinephrine-induced contractions of LCX and LAD arteries. Prazosin abolished norepinephrine-induced contractions of collateral-dependent LCX and normal LAD arteries, suggesting a dominant role of \(\alpha_2\)-receptor activation in these responses. We also determined that LCX arteries exhibit enhanced contractile responsiveness to the selective \(\alpha_1\)-adrenergic agonist phenylephrine compared with LAD arteries. Thus our data indicate that \(\alpha_1\)-adrenergic vasoconstriction is enhanced in large LCX arteries located distal to a chronic occlusion.

We investigated the role of nitric oxide in modulating \(\alpha_1\)-adrenergic receptor-mediated vasoconstriction by evaluating the effects of L-NAME on contractile responses of large LCX and LAD arteries to norepinephrine. In these experiments, inhibition of nitric oxide synthesis with L-NAME (100 \(\mu M\)) greatly enhanced norepinephrine-induced contractions of LAD arteries (\(P < 0.01\)) but had no significant effect on contractions of collateral-dependent LCX arteries. However, concentration-response curves for norepinephrine remained significantly different in LCX and LAD arteries treated with L-NAME (\(P < 0.01\)), which is consistent with the significant role of enhanced \(\alpha_2\)-adrenergic vasoconstriction. In contrast to effects of L-NAME on norepinephrine contractions, pretreatment with L-NAME did not alter the relative responsiveness of LCX and LAD arteries to \(K^+\) depolarization or PGF\(_{2\alpha}\). Thus our data indicate that decreased synthesis of nitric oxide is involved in the enhanced contractile responsiveness of LCX arteries to norepinephrine and that this impaired nitric oxide production is likely selective for \(\alpha_1\)-adrenergic stimulation.
mechanisms including \( Ca^- \) regulation are selectively upregulated in the smooth muscle of arteries located distal to coronary occlusion.

Collectively, our data indicate that the enhanced \( \alpha \)-adrenergic contractile responsiveness of large LCX arteries located distal to a chronic coronary occlusion involves alterations in \( \alpha \)-adrenergic responsiveness of the endothelium and smooth muscle. Comparison of the relative responsiveness to norepinephrine, an \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic agonist, and to phenylephrine, an \( \alpha_1 \)-adrenergic agonist, also supports the role of dual mechanisms underlying enhanced \( \alpha \)-adrenergic vasoconstriction of LCX arteries. Maximal norepinephrine-induced contractions of LCX arteries were more than sevenfold larger than contractions of LAD arteries. In contrast, maximal contractions of LCX arteries in response to phenylephrine were only 2.5-fold larger than contractions of LAD arteries. Thus the disparity between \( \alpha \)-adrenergic contractile responses of collateral-dependent LCX and normal LAD arteries is greater during combined stimulation of \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors than during stimulation of only \( \alpha_1 \)-receptors. Evaluation of the vasodilator effects of \( \alpha_2 \)-adrenergic stimulation on preconstricted arteries will be required to confirm a specific alteration in \( \alpha \)-adrenergic-mediated nitric oxide release in collateral-dependent arteries. However, all our data are consistent with the conclusion that enhanced \( \alpha_1 \)-adrenergic vasoconstriction and impaired \( \alpha_2 \)-adrenergic stimulation of nitric oxide synthesis from the endothelium underlie the enhanced \( \alpha \)-adrenergic contractile responsiveness of LCX arteries located distal to a chronic coronary occlusion.

Mechanisms of Impaired Relaxation

The vasodilator effects of adenosine are generally considered to be mediated by activation of adenylyl cyclase in vascular smooth muscle via coupling of A2 adenosine receptors to guanine nucleotide-binding stimulatory (Gs) proteins (13, 19, 21). Similarly, relaxation in response to isoproterenol involves coupling of \( \beta \)-adrenergic receptors to activation of adenylyl cyclase via G1 proteins (19, 28). This correlation in signaling pathways suggests that attenuated relaxation of LCX arteries to adenosine does not likely result from impairment of mechanisms common to the signal transduction of both agonists (i.e., Gs proteins, adenylyl cyclase, adenosine 3',5'-cyclic monophosphate-dependent protein kinase), given that \( \beta \)-adrenergic relaxation was not altered in LCX arteries. Alternatively, impaired adenosine-mediated responses of LCX arteries may result from decreased number of adenosine receptors or decreased efficiency of adenosine receptor coupling to signaling events. Our results do not discriminate between these possibilities. Relaxation of collateral-dependent LCX arteries to nitroprusside, a guanosine 3',5'-cyclic monophosphate-dependent vasodilator, was not significantly different from relaxation of normal LAD arteries. Unaltered relaxation responses to nitroprusside and isoproterenol indicate that the attenuated adenosine relaxation exhibited by LCX arteries does not result from a general functional impairment of smooth muscle.

Relation to Other Studies

This is the first report of altered responsiveness of large epicardial coronary arteries located distal to a chronic coronary occlusion. Potential mechanisms of altered vasomotor function of the collateral-dependent vasculature have been proposed previously by Sellke et al. (30). One possibility is that ischemia produced by coronary occlusion alters the responsiveness of arteries located in the collateral-dependent myocardial region. Indeed, ischemia and reperfusion have been shown to produce impairment of adenosine-mediated relaxation (12, 24). Other possible explanations of our findings may relate to the chronic decreases in distending pressure and/or reduced levels of fluid shear stress present in the collateral-dependent vasculature distal to occlusion. The pressure differential across the occlusion averages 90% during the time of Ameroid occlusion (27), gradually decreasing to 15–20% after significant development of the collateral circulation (30). In addition, as suggested by Sellke et al., the pulsatile nature of blood flow may be altered in the vasculature located distal to the occlusion. Recently, mechanical forces have been shown to be important regulators of gene expression in vascular cells. Interestingly, expression of \( \alpha \)-adrenergic receptors has been shown to be regulated by stretch in coronary vascular smooth muscle cells (22). However, the effects of the changes in hemodynamic forces resulting from coronary occlusion on \( \alpha \)-adrenergic and adenosine receptor populations in the distal vasculature remain to be elucidated.

Disruption of adrenergic innervation of the collateral-dependent vasculature by surgical dissection and/or myocardial ischemia could theoretically produce denervation supersensitivity in collateral-dependent arteries (14, 34). However, Roth and co-workers (26) previously determined that gradual Ameroid occlusion of the proximal LCX artery in dogs does not significantly alter the number of catecholamine-containing nerve terminals in the collateral-dependent vasculature or myocardium. Furthermore, we found that placement of the Ameroid occluder around the LCX artery without coronary occlusion did not produce alterations in responsiveness of collateral segments of the LCX (Fig. 2). Finally, sympathetic denervation supersensitivity is characterized by an increase in sensitivity to adrenergic agonists (14, 34). However, EC50 values for phenylephrine in LCX and LAD arteries were not significantly different. Thus our results are inconsistent with the phenomenon of denervation supersensitivity.

Implications

Convincing evidence exists for a significant role of \( \alpha \)-adrenergic coronary vasoconstriction in the initiation and aggravation of myocardial ischemia. In experimental models of coronary stenosis or during reductions in coronary perfusion pressure, adrenergic-mediated vasoconstriction detrimentally modulates metabolic coro-
nary dilation (8, 18). α-Adrenergic vasoconstriction distal to a coronary stenosis is sufficient to limit oxygen supply enough to impair myocardial function severely (8). A significant role of α-adrenergic coronary vasoconstriction in exercise-induced myocardial ischemia has been demonstrated in humans. Indeed, sympathetic activation by exercise has been shown to induce critical narrowing of stenotic coronary arteries sufficient to produce ischemic myocardial dysfunction and angina pectoris (7, 15). α-Adrenergic receptor antagonists reduce exercise-induced S-T segment depression (3, 11) and angina pectoris (3) and increase exercise capacity (11, 16) in patients with chronic stable angina. Kalsner and Richards (20) observed enhanced norepinephrine-induced contractions in coronary arteries from patients with coronary artery disease, suggesting that our findings in dogs may reflect pathological sequelae to experimental gradual coronary occlusion that are similar to those observed clinically in humans. Furthermore, augmented α-adrenergic vasoconstriction may have increased significance in the setting of impaired adenosine-mediated relaxation responses, producing a pathophysiological imbalance and, potentially, ischemia of collateral-dependent myocardium.

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