Endothelin antagonists block $\alpha_1$-adrenergic constriction of coronary arterioles

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1Center for Anesthesiology Research, The Cleveland Clinic Foundation, Cleveland, Ohio 44195; 2Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226-0509; and 3Department of Medical Physiology, Texas A&M University Health Science Center, Microcirculation Research Institute, College Station, Texas 77843

Defily, David V., Yasuhiro Nishikawa, and William M. Chilian. Endothelin antagonists block $\alpha_1$-adrenergic constriction of coronary arterioles. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1028–H1034, 1999.—We have previously observed that intracoronary administration of the $\alpha_1$-adrenergic agonist phenylephrine (PE) over a period of minutes induced both an immediate and long-lasting (2 h) vasoconstriction of epicardial coronary arterioles. Because it is unlikely that $\alpha_1$-adrenergic constriction would persist for hours after removal of the agonist, this observation supports the view that another constrictor(s) is released during $\alpha_1$-adrenergic activation and induces the prolonged vasoconstriction. Therefore, we hypothesized that the prolonged microvascular constriction after PE is due to the production of endothelin (ET). We focused on ET not only because this peptide produces potent vasoconstriction but also because its vasoconstrictor action is characterized by a long duration. To test this hypothesis, the diameters of coronary arterioles (~222 µm) in the beating heart of pentobarbital-anesthetized dogs with stroboscopic intravital microscopy were measured during a 15-min intracoronary infusion of PE (1 µg·kg$^{-1}$·min$^{-1}$) and at 15-min intervals for a total of 120 min. All experiments were performed in the presence of $\beta$-adrenergic blockade with propranolol. At 120 min, arterioles in the PE group were constricted (~23 ± 9% change in diameter vs. baseline). Pretreatment with the ET-converting enzyme inhibitor phosphoramidon or the ETA-receptor antagonist FR-139317 prevented the sustained constriction (P < 0.01 vs. PE). Pretreatment with the selective $\alpha_1$-adrenergic agonist prazosin (Prz) also prevented the sustained constriction (P < 0.01 vs. PE) but Prz given 60 min after PE infusion did not (~13 ± 3%). In the aggregate, these results show that vasoconstriction of epicardial coronary arterioles via $\alpha_1$-adrenergic activation is blocked by an ET antagonist and an inhibitor of its production. From these data, we conclude that $\alpha_2$-adrenergic activation promotes the production and/or release of ET, which produces or facilitates microvascular constriction of epicardial canine coronary arterioles.

 Coronary microcirculation; coronary circulation; phenylephrine

ACTIVATION of $\alpha_1$-adrenergic receptors in vivo produces constriction of coronary microvessels (1, 4, 9), increases coronary resistance (7, 23), and decreases coronary blood flow (17). The obvious and accepted conclusion of these studies is that the constriction is due to activation of $\alpha_1$-adrenergic receptors on coronary vascular smooth muscle. Although this conventional explanation is accepted by virtually all investigators, some observations suggest that $\alpha_1$-adrenergic influences on coronary microvessels may be more complex. For example, Muntz et al. (18) used an autoradiographic technique to measure the distribution of $\alpha_1$-adrenergic receptors in the myocardium and found that the number of receptors on coronary arterioles was about at background levels. We also reported that isolated coronary arterioles do not respond to $\alpha_1$-adrenergic activation, in contrast to arterioles isolated from skeletal muscle or even coronary venules (10). Finally, in an investigation of the interaction between $\alpha_2$- and $\alpha_1$-adrenergic constriction and the endogenous levels of adenosine, we observed that intracoronary infusion of high doses of the $\alpha_1$-adrenergic agonist phenylephrine resulted in both an immediate and prolonged constriction of coronary arterioles in vivo lasting up to 2 h (4). Taken together, these results challenge the conclusion that $\alpha_1$-adrenergic receptor activation on coronary smooth muscle is causal for coronary arteriolar or resistance vessel vasoconstriction.

If microvascular constriction is not directly due to $\alpha_1$-adrenergic activation on coronary smooth muscle, then other mechanisms must be proposed. The time course of the prolonged constriction described above (4) may offer evidence of a possible mechanism. Although it is unlikely that $\alpha_1$-adrenergic constriction would persist for up to 2 h after removal of the agonist, this is similar to the time course reported for the vasoconstrictor action of endothelin (3, 16, 24). Furthermore, there are many studies that have examined the potent coronary vascular effects of endothelin in vivo and in vitro (3, 8, 13, 15), but there is little knowledge about its role in the regulation of coronary microvascular tone.

Therefore, the objective of this study was to test the hypothesis that intracoronary infusion of the $\alpha_1$-adrenergic agonist phenylephrine induces a constriction of coronary arterioles that is mediated by endothelin. To investigate this hypothesis, we measured the diameter of epicardial coronary arterioles after intracoronary infusion of phenylephrine. The role of endothelin was tested by blocking its production with phosphoramidon and its action with a selective ETA-receptor antagonist.

METHODS

General Preparation

Adult beagles (8–12 kg) were anesthetized with pentobarbital sodium (35 mg/kg iv), intubated, and ventilated with room
A femoral artery and vein were catheterized for measurement of arterial pressure, arterial blood gases and pH, and fluid or drug administration. A catheter was inserted into the carotid artery and advanced into the left ventricle (LV) for measurement of LV pressure and the first derivative of LV pressure with respect to time (LV dp/dt). The heart was exposed via a left thoracotomy through the fifth intercostal space, and the heart was suspended in a pericardial cradle. A 24-gauge Teflon catheter (Surflo, intravenous catheter) was placed in the proximal left circumflex coronary artery and secured to the adventitia with 4-0 suture, for measurements of coronary artery pressure and intracoronary administration of drugs and fluorescent dye. The cross-sectional area of the Teflon catheter (0.37 mm²) constitutes a minor fraction of the lumen of the cannulated vessel because most vessels are in the 2.5- to 3-mm range, with cross-sectional areas of 4.9 mm² or larger.

After these procedures, the animal was ventilated on a high-frequency jet ventilator (supplemented with 60% N₂, 40% O₂ at a pressure of 9-20 psi) synchronized to the cardiac cycle. A pressure regulator connected to a solenoid valve was triggered from the LV dp/dt and remained open for 30-40 ms each cardiac cycle. This jet ventilation results in low tidal volume ventilation once per cardiac cycle at the same point each cardiac cycle. This jet ventilation results in low tidal volume that were maintained within normal limits (pH 7.35-7.45, Pco₂ 30-40 mmHg, Pao₂ > 100 mmHg). Blood gases and pH were varied with slight adjustments in the position of the tracheal cannula, the pressure driving the ventilator, the duration of inflation, and the administration of sodium bicarbonate.

To minimize cardiac motion, four small pins attached to a single support rod were inserted into the myocardium. The pins (22 gauge) were oriented so that lateral movement of the microvascular field was restricted to 600–1,000 µm, and vertical movements were nearly abolished. The majority of studies would be impossible without the pinning of the myocardium because the vessels would move in and out of the field of view and therefore in and out of focus. Vessels within 1 cm of the point of insertion or exit of the pins were not studied. It has been determined that both resting and maximal myocardial blood flow are not altered in “restrained” myocardium because the vessels would move in and out of the field of view and therefore in and out of focus. Vessels within 1 cm of the point of insertion or exit of the pins were not studied. It has been determined that both resting and maximal myocardial blood flow are not altered in “restrained” myocardium because the vessels would move in and out of the field of view and therefore in and out of focus. Vessels within 1 cm of the point of insertion or exit of the pins were not studied.

Intravital Microscopy

The intravital microscope system consists of a Leitz Ploemopak (Wild Leitz, Rockleigh, NJ) mounted on a vertical support over an x-y adjustable table. The use of the x-y adjustable table allowed for fine movements of the position of the heart within the field of view. The Ploem system was used with filters for fluorescence microscopy. A total magnification of the video image of approximately X 200 was achieved by a combination of the microscope objective (Leitz L10 X 10, numerical aperture 0.22), a X 10 magnification eyepiece, and a video display. The resolution of this configuration is ~2 µm. Illumination of the epicardial surface of the LV was accomplished with a xenon stroboscopic light source (Chadwick-Helmuth, El Monte, CA) synchronized to LV dp/dt. This resulted in stroboscopic illumination once per cardiac cycle. A computer (Macintosh Quadra 950) received input from the LV dp/dt and subsequently triggered the strobe at the same point in time (late diastole) within the cardiac cycle. With this system, the heart and microvasculature appear to be “fixed” simply because the epicardium is in view for only one video field (16 ms) at the same time in each cardiac cycle.

Video images of coronary microvessels were obtained with an intensified charged-coupled device camera (model 5515, Cohn, San Diego, CA) and recorded with a frame digitizer (Neotech). Control of video acquisition was achieved with LabVIEW software (National Instruments, Austin, TX). This involved digitizing a series of video frames, evaluating them for focus and other aspects of quality, and then storing them on the computer. Microvascular diameter measurements were made at a later time on the Macintosh computer with image-processing software (Image 1.57, National Institutes of Health, Bethesda, MD).

The microvasculature was visualized with fluorescence video microscopy. Small intracoronary bolus injections (~50-100 µl) of FITC-labeled bovine albumin (Sigma, St. Louis, MO) were made via the coronary catheter. Measurement of microvascular caliber during fluorescence microscopy provides data on the internal diameter of the blood vessel, because the blood vessel wall is not illuminated by the fluorochrome. Because the bolus of FITC arrived at the arteries and arterioles first, we could distinguish the difference between arterioles and venules. The existence of a well-defined anatomic landmark (i.e., branching point) was the major criterion used in the selection of a specific arteriole. This ensured that the diameter was measured at the same point on the vessel throughout all experimental interventions.

Experimental Protocols

For each protocol, baseline hemodynamic and microvascular diameter measurements were made in the presence of β-adrenergic receptor blockade with propranolol (1 mg/kg). After these measurements, one of the following protocols was followed.

Group 1: Phenylophrine. Selective α₁-adrenergic receptor activation was achieved by intracoronary infusion of phenylephrine (1 µg·kg⁻¹·min⁻¹; n = 5 dogs). Hemodynamic and microvascular diameter measurements were made at 5 and 15 min after the start of the phenylephrine infusion. The phenylephrine infusion was stopped after 15 min, and microvascular diameter and hemodynamic measurements were made at 15-min intervals for the next 105 min.

Group 2: Rauwolscine-treated norepinephrine. Selective α₁-adrenergic receptor activation was achieved by intracoronary infusion of norepinephrine (0.2 µg·kg⁻¹·min⁻¹; n = 5 dogs) after pretreatment with an intravenous injection of the selective α₂-adrenergic antagonist rauwolscine (0.2 mg/kg). The norepinephrine infusion was stopped after 15 min.

Group 3: Prazosin. Selective α₁-adrenergic receptor activation with phenylephrine was achieved as described for group 1 after a 10-min application of prazosin (0.75 mg/kg iv; n = 6 dogs). Also, as described for group 1, measurements were continued until 120 min after the initial phenylephrine infusion.

Group 4: Prazosin 60 min after phenylephrine. Selective α₁-adrenergic receptor activation with phenylephrine was achieved as described for group 1. After the 60-min measurements, α₁-adrenergic receptor blockade was achieved with prazosin (0.75 mg/kg iv; n = 5 dogs). Measurements were continued until after the 120-min time period.

Group 5: Phosphoramidon. Selective α₁-adrenergic receptor activation with phenylephrine was achieved as described for group 1. A bolus dose (2 mg/kg iv; n = 5 dogs) of the endothelin-converting enzyme inhibitor phosphoramidon was given 10 min before the start of phenylephrine infusion. Also,
Hemodynamics and Baseline Diameters

Mean aortic pressure, mmHg

<table>
<thead>
<tr>
<th>Group</th>
<th>Prdn</th>
<th>Prz60</th>
<th>Prz</th>
<th>PE</th>
<th>NE</th>
<th>ETx</th>
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<tr>
<td>C</td>
<td>93 ± 8</td>
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<td>96 ± 3</td>
<td>139 ± 17</td>
<td>93 ± 13</td>
<td>96 ± 11</td>
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<td>PE</td>
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<tr>
<td>Prdn</td>
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<td>118 ± 3</td>
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<tr>
<td>ETx</td>
<td>93 ± 5</td>
<td>98 ± 8</td>
<td>118 ± 11</td>
<td>101 ± 9</td>
<td>95 ± 5</td>
<td>88 ± 6</td>
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Table 1. Hemodynamics

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<th>30</th>
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<td>PE</td>
<td>91 ± 7</td>
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<td>106 ± 6</td>
<td>104 ± 5</td>
<td>96 ± 5</td>
<td>101 ± 7</td>
</tr>
<tr>
<td>NE</td>
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<td>99 ± 9</td>
<td>103 ± 10</td>
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<tr>
<td>Prz</td>
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<td>81 ± 6</td>
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<td>Prz60</td>
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<td>128 ± 17</td>
<td>120 ± 9</td>
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<td>114 ± 11</td>
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<td>101 ± 9</td>
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<td>88 ± 6</td>
<td>86 ± 6</td>
<td>85 ± 6</td>
<td>87 ± 4</td>
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Values are means ± SE. n = 5 dogs for phenylephrine (PE), norepinephrine (NE), prazosin administered after 60-min measurement (Prz60), phophoramid (Prdn), and endothelin-receptor antagonist FR-139317 (ETx); n = 6 dogs for prazosin (Prz). C, control. See Experimental Protocols for details. *P < 0.05 vs. PE group (repeated-measures ANOVA).

as described for group 1, measurements were continued until 120 min after the initial phenylephrine infusion.

Group 6: FR-139317. Selective α₁-adrenergic receptor activation with phenylephrine was achieved as described for group 1. Intravenous infusion of the ETα₁-receptor agonist FR-139317 (10 µg·kg⁻¹·min⁻¹; n = 5 dogs) was begun 10 min before the start of phenylephrine infusion and was continued until after the 120-min measurements were made.

Control studies were performed to determine the time course of endothelin-induced constriction of coronary arterioles. A suffusion of endothelin (10 nM) onto the surface of the heart was continued for 15 min, and measurements of arteriolar diameters were made every 15 min as described for group 1 (n = 2 dogs). In one additional dog, an intravenous infusion of FR-139317 (10 µg·kg⁻¹·min⁻¹) was given for 15 min during the endothelin suffusion. The endothelin suffusion was continued for another 30 min. Measurements were made for a total of 120 min as described for group 1.

Drugs

Propranolol, phenylephrine, norepinephrine, and prazosin were obtained from Sigma. Rauwolsine was obtained from Research Biochemicals International (Natick, MA). FR-139317 was a gift from Dr. Terry Opengorth of Abbott Laboratories (Abbott, IL).

Data Analysis

Hemodynamic data (LV pressure, LV dP/dt, mean aortic pressure, mean coronary artery pressure, and heart rate) were digitized, displayed, stored, and analyzed on a Macintosh computer (Quadra 950) with LabVIEW software or a CODAS data-acquisition system.

The data are presented as means ± SE. Diameter measurements of the same vessel from two to eight video frames were averaged for each diameter measurement and thus considered a datum (n = 1). Typically the variation within these frames is <3%. Microvascular diameters during α₁-activation were expressed as a percent change from baseline and were calculated as follows:

%change = [(D - D₀)/D₀] × 100

where D is diameter and D₀ is baseline diameter. To test for significant differences in microvascular diameter and hemodynamics at all doses of the agonists, a two-way repeated measures ANOVA was used (StatView 4.1, Abacus Concepts, Berkeley, CA). Post hoc comparisons were made with Scheffe's F test. Significance was set at the 5% level.

RESULTS

Hemodynamics and Baseline Diameters

Mean aortic pressure, coronary artery pressure, and heart rate data are presented in Table 1 for all time points. There was no significant difference in hemodynamics between the phenylephrine group and any other group. Baseline diameter data for all vessels are presented in Table 2. All vessels used in this study fell

Table 2. Baseline microvascular diameters

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Vessels</th>
<th>Diameter, µm</th>
</tr>
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<tbody>
<tr>
<td>PE</td>
<td>13</td>
<td>87 ± 6</td>
</tr>
<tr>
<td>NE</td>
<td>12</td>
<td>92 ± 8</td>
</tr>
<tr>
<td>Prz</td>
<td>19</td>
<td>97 ± 9</td>
</tr>
<tr>
<td>Prz60</td>
<td>21</td>
<td>96 ± 7</td>
</tr>
<tr>
<td>Prdn</td>
<td>14</td>
<td>92 ± 8</td>
</tr>
<tr>
<td>ETx</td>
<td>16</td>
<td>81 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE. No significant difference was found among groups with ANOVA. See Table 1 for abbreviations, and see Experimental Protocols for details.
within the size range of 49–222 µm in diameter. These data indicate that the baseline diameters of microvessels in all groups were not significantly different from those in the phenylephrine group.

Microvascular Diameter

During the intracoronary infusion of 1.0 µg·kg\(^{-1}\)·min\(^{-1}\) phenylephrine, coronary arterioles constricted by \(-9\%\). After the 15-min infusion, measurements of microvascular diameter for another 105 min (120 min total) revealed a significant progressive vasoconstriction, resulting in \(-23 \pm 9\%\) change in diameter relative to baseline at 120 min (Fig. 1). Similar arteriolar vasoconstriction was observed after \(\alpha_1\)-adrenergic activation achieved by intracoronary infusion of norepinephrine (0.2 µg·kg\(^{-1}\)·min\(^{-1}\)) in the presence of \(\alpha_2\)-adrenergic blockade with rauwolscine (0.2 µg/kg) (\(-10 \pm 3\%\) change in diameter, not significant vs. phenylephrine; Fig. 1). Blockade of \(\alpha_1\)-adrenergic receptor activation with prazosin (0.75 mg/kg iv) before phenylephrine infusion prevented the sustained constriction observed at 120 min (0 \pm 2\% with phenylephrine alone (\(P < 0.01\) vs. phenylephrine). However, the addition of prazosin 45 min after the phenylephrine infusion and after the 60-min measurement did not prevent the constriction at 120 min (\(-13 \pm 3\\%\). Our data suggest that the sustained vasoconstriction after intracoronary infusion of phenylephrine is mediated by endothelin-1, because antagonism of \(\text{ET}_A\) receptors or inhibition of endothelin-converting enzyme resulted in a significant reduction or elimination of the vasoconstriction at 120 min. This was accomplished by two different mechanisms (Fig. 2). First, inhibiting the conversion of preproendothelin to endothelin-1 with the converting enzyme inhibitor phosphoramidon completely inhibited the sustained vasoconstriction of arterioles (\(-1 \pm 2\%\) change in diameter, \(P < 0.01\) vs. phenylephrine). Second, constant infusion of the \(\text{ET}_A\)-receptor antagonist FR-139317 significantly attenuated the vasoconstriction at 120 min (\(-6 \pm 3\%\) change in diameter, \(P < 0.01\) vs. phenylephrine).

Control studies (Fig. 3) demonstrate that suffusion of 10 nM endothelin-1 on the surface of the heart results in a similar time course and amplitude of arteriolar vasoconstriction (\(-14 \pm 3\%\) change in diameter) as that observed after intracoronary infusion of phenylephrine. Endothelin suffusion during infusion of the endothelin receptor antagonist, however, resulted in a vasodilation (11 \pm 2\% change in diameter, \(P < 0.05\) vs. endothelin suffusion alone). Removal of the endothelin antagonist in the presence of endothelin suffusion again resulted in a vasoconstriction, which by 120 min was not different from that obtained with endothelin alone (\(-15 \pm 5\) and \(-14 \pm 3\%\).

DISCUSSION

Summary

In this study, we have made the novel observation that after activation of \(\alpha_1\)-adrenergic receptors, epicardial coronary arterioles progressively constricted for at least 105 min after infusion of the agonist was stopped. \(\alpha_1\)-Adrenergic activation was achieved by either intracoronary infusion of phenylephrine or intracoronary infusion of norepinephrine in the presence of the \(\alpha_2\)-adrenergic receptor antagonist rauwolscine. Both procedures resulted in arteriolar constriction with a similar time course and amplitude. Blockade of \(\alpha_1\)-adrenergic receptor activation with the selective \(\alpha_1\)-adrenergic receptor antagonist prazosin given before the agonist
also prevented the arteriolar constriction observed at 120 min, demonstrating a dependence on α₁-adrenergic activity, at least for the initiation of the response. However, when prazosin was administered 1 h after the agonist infusion, the constriction was not attenuated. This finding suggests that the long-lasting constriction is independent from direct activation of α₁-adrenergic receptors on coronary smooth muscle. The sustained arteriolar constriction after phenylephrine infusion, however, was prevented by pretreatment with phosphoramidon, an inhibitor of the conversion from preproendothelin to endothelin, or by constant intravenous infusion of the selective ETA-receptor antagonist FR-139317. These results suggest that coronary arteriolar constriction after α₁-adrenergic activation stimulation may result from the stimulation and release of endothelin.

Critique of Experimental Methods

To consider our hypothesis of an α₁-adrenergic-mediated increase in endothelin, we must assume that the local levels of endothelin are increased. While plasma endothelin levels are extremely low, circulating endothelin levels would likely not accurately reflect the concentration at the microvascular wall (16). The functional evidence presented here demonstrates a role of endothelin in the prolonged constriction of coronary arterioles. This was achieved by two different methods of inhibiting the pressor responses of endothelin-1. In one group, we performed the phenylephrine infusion and all subsequent measurements during intravenous infusion of the specific ETₐ-receptor antagonist FR-139317. As demonstrated in Fig. 3, the dose of FR-139317 used (10 µg·kg⁻¹·min⁻¹) was sufficient to prevent the coronary arteriolar pressor response of 10 nM endothelin-1 suffusion over the surface of the heart. Lamping et al. (15) have previously shown that this dose of endothelin produces a substantial constriction of coronary arterioles when suffused on the surface of the heart. In another group, animals were pretreated with the endothelin-converting enzyme inhibitor phosphoramidon (25). Phosphoramidon, which prevents the conversion of Big endothelin-1 to endothelin-1, has been shown previously to prevent the pressor response of Big endothelin-1, the precursor of endothelin-1 (25). Whereas the use of the endothelin-1 receptor antagonist prevents the binding of endothelin-1 to receptors on the vascular smooth muscle and hence prevents endothelin-induced constriction, phosphoramidon prevents the formation of endothelin-1, the active form of the peptide.

In all cases, intracoronary rather than intravenous infusion of phenylephrine or norepinephrine was used to reduce peripheral hemodynamic changes. Slight but statistically insignificant increases in arterial and coronary artery pressure had occurred by the end of the 15-min phenylephrine infusion, but these had quickly returned to control levels. At the end of the 120-min measurement period, pressures were at baseline levels even though coronary arterioles were significantly constricted. Pressures were decreased at most middle time points in the animals receiving prazosin. If anything, a decrease in pressure and the associated reduction in myocardial oxygen demands would produce constriction of arterioles; however, we observed a trend toward vasodilation. Importantly, at 120 min when differences among hemodynamics in the various groups were not evident, differences in diameters were observed. These results suggest that the constriction was caused by the various agonists and that the blockade of constriction was due to antagonists rather than secondary changes in tone associated with hemodynamic perturbations.

Physiological Implications

The importance of this study is that it provides further insight into regulation of the coronary microcirculation by the 21-amino acid peptide endothelin. An observation that has perplexed us is that although α₁-adrenergic constriction of coronary arterioles occurs in vivo (1, 4), it cannot be repeated in vitro (10). These data lead to the speculation that α₁-adrenergic vasoconstriction of coronary arterioles in vivo may be mediated or at least facilitated by the production and release of endothelin.

Although the data presented here suggest that the sustained coronary arteriolar vasoconstriction is mediated by endothelin, we can only speculate as to the origin of the endothelin. The initial description of endothelin by Yanagisawa et al. (24) described its origin from endothelial cells. There is also evidence in the literature for the stimulation of endothelin or preproendothelin secretion from cultured endothelial cells by vasoconstrictive hormones (5, 6, 24). Specifically, incubations of cultured bovine endothelial cells with 12-O-tetradecanoylphorbol 13-acetate, ionomycin, thrombin, ANG II, and arginine vasopressin have all resulted in a significant and dose-dependent increase in endothelin secretion (6). Kohno et al. (14) had also recently reported a stimulatory effect of epinephrine on the release of endothelin from cultured porcine endothelial cells. The stimulatory effect of epinephrine was blocked by the α₂-adrenergic antagonist fenoldopam but not yohimbine, an α₁-adrenergic antagonist, indicating an α₂-adrenergic-mediated effect. Indeed, this stimulatory effect could be blocked by the α₁-adrenergic antagonist prazosin but not yohimbine, an α₂-adrenergic antagonist, indicating that selective α₁-adrenergic activity is responsible (19). Although the 3- to 6-h time course of these studies was somewhat longer than that of our study, Yanagisawa et al. (24) had also reported an increase in endothelial cell preproendothelin mRNA within 1 h of treatment with epinephrine and other agents, a time course that follows closely to that of our findings. Similarly, in spontaneously hypertensive rats, 5-h perfusion of mesenteric arteries with ANG II produced a potentiation of norepinephrine-induced contractions that was inhibited by phosphoramidon or an anti-endothelin antibody (5). Preproendothelin mRNA levels were also increased in endothelial cells isolated from these spontaneously hypertensive rats after exposure to ANG II. Although this study only suggests that this increase in endothelin levels occurs in spontaneously hypertensive rats...
and only after a 4- to 5-h infusion of ANG II, similar studies have not yet been performed in the dog coronary microvascular bed.

Although endothelin was originally considered an endothelial cell peptide, it is also possible that the α-adrenergic-mediated endothelin production could originate in myocardial cells. Recent studies have demonstrated the presence of ET-1 mRNA in both cultured adult rat (11) and neonatal rat (12) cardiomyocytes. In these studies, cultured ventricular myocytes treated with noradrenaline were shown to produce significant increases in ET-1 mRNA after as little as 1 h of incubation (11). Similarly, rat neonatal ventricular myocytes that were shown to express ET-1 mRNA in unstimulated conditions produced significant increases at 1 h when they were stimulated with the α1-adrenergic agonist phenylephrine (12). These results add additional insight into our observations and other reports in the literature. Within this context, for α1-adrenergic receptor activation to mediate endothelin production from myocardial cells, there must be evidence of α-adrenergic receptors on myocardial cells. Autoradiographic studies have demonstrated the presence of α1-adrenergic receptors in both rat (18) and feline (20) myocardium. Indeed, regions of closely arranged cardiac myocytes contained three to four times more [3H]prazosin than did regions composed of coronary arterioles in feline myocardium (20). Likewise, a substantially higher density of α1-adrenergic receptors was also noted in rat myocardium than in rat coronary arterioles (18). Rat coronary arterioles also contained a lower density of receptors and lower affinity for the [3H]prazosin than did rat renal arterioles (18). However, the general impression from these studies (18, 20) is that coronary arterioles possess some α1-adrenergic receptors but that substantially more are located on cardiac myocytes. In addition, the study of Jones et al. (10) may also suggest a myocardial origin of α1-adrenergic receptor-mediated release of endothelin. In that study, the authors were unable to demonstrate α2-adrenergic mediator constricted of isolated, buffer-perfused coronary arterioles. This observation also supports the idea that endothelin is not produced by the endothelium during α1-adrenergic activation, because if it were, then constrictions would have occurred in isolated arterioles. This, however, was not the observation. In another recent study from our laboratory, it was observed that isolated coronary arterioles that did not constrict to phenylephrine directly did constrict in the presence of aliquots of supernatant from phenylephrine-treated cardiac myocytes (22). The phenylephrine dose-dependent constriction was also blocked by an ETA antagonist or by treating the myocytes with prazosin. However, prazosin treatment of the arterioles did not block constriction produced by the supernatant. In the aggregate, these results provide evidence showing that cardiac myocytes may be the target for α1-adrenergic agonists, and on activation they may release endothelin. We speculate that the α1-adrenergic-induced release of endothelin is balanced by many factors, e.g., oxygenation and adenosine, so as not to cause excessive constriction.

We are compelled to point out that our observations are confined to the epicardial microcirculation, and extension of our findings to intramural microvessels must be made with caution. We make this statement because investigators do not routinely report large increases in coronary resistance or decreases in coronary blood flow for long periods of time after α1-adrenergic activation. Because overall coronary flow reflects principally the intramural microcirculation, we must be cautious about our observations. However, we can affirm that α1-adrenergic constriction of epicardial coronary arterioles can be blocked by ETA antagonists or by inhibition of endothelin-converting enzyme.

In conclusion, we speculate from these data that a component of α1-adrenergic constriction of epicardial coronary arterioles in vivo is mediated by endothelin. We suggest that α1-adrenergic receptor activation promotes endothelin production from the myocardium, which in turn produces coronary arteriolar constriction. These results help explain the many disparate findings about α1-adrenergic activation and the resulting coronary arteriolar constriction. Specifically, the dissimilar findings of constriction in vivo but not in vitro, the paucity or lack of α1-adrenergic receptors on coronary arterioles, and the long duration of α1-adrenergic constriction in coronary arterioles, which is inconsistent with the actions of α1-adrenergic activation but totally consistent with the coronary vascular effects of endothelin, may be explained by these results. The data also provide new insights into the role of endothelin in the modulation of coronary blood flow. The physiological importance of this mechanism and the source of endothelin-1 in the response remain unknown. We speculate that the production of endothelin by the myocardium represents an adaptation to integrate neurohumoral stimulation with myocardial metabolism. These “opposing” factors have their actions integrated at the level of the myocyte, and the consequence on coronary resistance vessels is the net effect of production of these constrictors and dilators by the cardiac myocyte.

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