α-Adrenoceptor-mediated coronary vasoconstriction is augmented during exercise in experimental diabetes mellitus

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Setty, Srinath, Wei Sun, Rodolfo Martinez, H. Fred Downey, and Johnathan D. Tune. α-Adrenoceptor-mediated coronary vasoconstriction is augmented during exercise in experimental diabetes mellitus. J Appl Physiol 97: 431–438, 2004. First published February 20, 2004; 10.1152/japplphysiol.01122.2003.—This study tested whether α-adrenoceptor-mediated coronary vasoconstriction is augmented during exercise in diabetes mellitus. Experiments were conducted in dogs instrumented with catheters in the aorta and coronary sinus and with a flow transducer around the circumflex coronary artery. Diabetes was induced with alloxan monohydrate (n = 8, 40 mg/kg iv). Arterial plasma glucose concentration increased from 4.7 ± 0.2 mM in nondiabetic, control dogs (n = 8) to 21.4 ± 1.9 mM 1 wk after alloxan injection. Coronary blood flow, myocardial oxygen consumption (MVO₂), aortic pressure, and heat rate were measured at rest and during graded treadmill exercise before and after infusion of the α-adrenoceptor antagonist phenolamine (1 mg/kg iv). In untreated diabetic dogs, exercise increased MVO₂ 2.7-fold, coronary blood flow 2.2-fold, and heat rate 2.3-fold. Coronary venous PO₂ fell as MVO₂ increased during exercise. After α-adrenoceptor blockade, exercise increased MVO₂ 3.1-fold, coronary blood flow 2.7-fold, and heat rate 2.1-fold. Relative to untreated diabetic dogs, α-adrenoceptor blockade significantly decreased the slope of the relationship between coronary venous PO₂ and MVO₂. The difference between the untreated and phenolamine-treated slopes was greater in the diabetic dogs than in the nondiabetic dogs. In addition, the decrease in coronary blood flow to intracoronary norepinephrine infusion was significantly augmented in anesthetized, open-chest, β-adrenoceptor-blocked diabetic dogs compared with the nondiabetic dogs. These findings demonstrate that α-adrenoceptor-mediated coronary vasoconstriction is augmented in alloxan-induced diabetic dogs during physiological increases in MVO₂.

coronary blood flow; myocardial oxygen consumption

EARLIER STUDIES HAVE SHOWN that Type 1 diabetes mellitus increases myocardial α-adrenoceptor sensitivity but decreases the overall number of α-adrenoceptor binding sites (27, 32, 48). These changes correspond with enhancement of α-adrenoceptor-mediated increases in cardiac function, i.e., maximal change in pressure over time and developed tension, after administration of a selective α-agonist to acute diabetic animals (2, 14, 24). Diabetes may also alter α-adrenoceptor-mediated coronary vasoconstriction because stimulation of the cardiac plexus induces vasoconstriction in alloxan-induced diabetic dogs but vasodilation in normal, control dogs (31). In addition, studies from isolated coronary microvessels found that arterioles from diabetic rats constrict to norepinephrine superfusion, whereas arterioles from nondiabetic rats dilate (40). These findings suggest that diabetes may significantly augment α-adrenoceptor-mediated coronary vasoconstriction; however, they do not address whether this alteration contributes to the physiological impairment of the balance between coronary blood flow and myocardial metabolism induced by diabetes (47). Characterizing the mechanisms of diabetic coronary dysfunction is important because it could help explain the increased incidence of myocardial ischemia, infarction, and sudden cardiac death among patients with diabetes (18, 22).

The present investigation was designed to test the hypothesis that α-adrenoceptor-mediated coronary vasoconstriction is elevated in diabetic animals during increases in myocardial oxygen demand. Experiments were conducted in chronically instrumented dogs with and without α-adrenoceptor blockade with phenolamine (1 mg/kg iv). These experiments were performed in both nondiabetic and diabetic dogs at rest and during graded treadmill exercise. Exercise was used because it is an excellent physiological stimulus that dramatically increases cardiac work and myocardial metabolism. Diabetes was induced with alloxan monohydrate (40 mg/kg iv) (46, 47). Additional experiments were also conducted in anesthetized, open-chest, β-adrenoceptor-blocked (propranolol 2 mg/kg iv) dogs to determine whether norepinephrine-mediated coronary vasoconstriction is augmented in alloxan-induced diabetic dogs.

METHODS

Studies Conducted in Chronically Instrumented Dogs

Surgical preparation. This investigation was approved by the Institutional Animal Care and Use Committee and was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 1996). Experiments were performed on 13 adult mongrel dogs of either sex (weighing 16–37 kg) taught to run on a motorized treadmill. Eight of these dogs served in two or more of the experimental groups. Preanesthesia (acepromazine 0.03 mg/kg im) was administered 30 min before induction of anesthesia with thiopental sodium (5 mg/kg iv). After endotracheal intubation, a surgical plane of anesthesia was maintained by mechanical ventilation with 1–3% isoflurane gas plus supplemental oxygen. With the use of sterile technique, a left lateral thoracotomy was performed in the fifth intercostal space. A custom-made, coextruded polyurethane catheter (Putnam Plastics) was implanted in the de-
ascending thoracic aorta to measure aortic blood pressure and to obtain arterial blood samples (44–46). A second polyurethane catheter was placed in the coronary sinus via a purse-string suture in the right atrial appendage for coronary venous blood sampling. The circumflex coronary artery was dissected free, and a flow transducer (see Pressure and flow measurements) was placed around the artery. A chest tube was placed to evacuate the pneumothorax, and the chest was closed in layers. The catheters and the flow transducer wire were tunneled subcutaneously and exteriorized between the scapulae. The incision was infiltrated with 2.5% bupivacaine, and Buprenex (0.3 mg im) was administered to minimize postoperative pain. Clavamox (6.25 mg/lb) and aspirin (81 mg) were administered twice daily for 10 days after surgery. Nylon jackets (Alice King Chatham) were placed on the animals to protect the catheters and the flow transducer wire. A closed in layers. The catheters and the flow transducer wire were tunneled subcutaneously and exteriorized between the scapulae. The incision was infiltrated with 2.5% bupivacaine, and Buprenex (0.3 mg im) was administered to minimize postoperative pain. Clavamox (6.25 mg/lb) and aspirin (81 mg) were administered twice daily for 10 days after surgery. Nylon jackets (Alice King Chatham) were placed on the animals to protect the catheters and the flow transducer wire. A elastomeric balloon pump (Access Technologies) was connected to the coronary sinus catheter so heparinized saline (5 U/ml) could be continuously infused at 5 ml/h. The aortic catheter was flushed daily and filled with heparinized saline (1,000 U/ml). The animals were allowed at least 7 days for recovery before the experiments were conducted.

**Pressure and flow measurements.** A coexruded polyurethane catheter was implanted in the aorta, so that a high-fidelity Mikro-tipped pressure transducer (3F, Millar Instruments) could be inserted through it at the time of the experiment to measure aortic blood pressure (44–46). This pressure transducer was introduced into the polyurethane aortic catheter through a Tuohy-Borst hemostatic control valve (Mallinckrodt Medical), which allowed arterial blood samples to be withdrawn while maintaining a fluid tight seal around the Millar catheter.

Phasic and mean coronary blood flows were continuously measured throughout the experimental protocol (see Experimental protocols) with an ultrasonic, flow transducer (Transonic Systems). After experiments were completed, the animals were euthanized with pentobarbital sodium, and the perfusion space, and the heart was suspended in a pericardial cradle. The left coronary arterial perfusion system and right femoral vein for intravenous administration of supplemental anesthetic, sodium bicarbonate, and propranolol. A catheter was also placed in the left femoral artery to measure arterial blood pressure.

A left lateral thoracotomy was performed in the fifth intercostal space, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was isolated distal to its first major branch. Propranolol (2 mg/kg iv) was then infused to inhibit norepinephrine-mediated activation of β-adrenoceptors. After heparinization (500 U/kg iv), the LAD was cannulated with a stainless steel cannula (3.0 mm OD, 2.2 mm ID) connected to the extracorporeal perfusion system. Coronary perfusion pressure was measured through a saline-filled catheter advanced to the orifice of the LAD cannula. The coronary perfusion pressure was maintained at 100 mmHg throughout the entire protocol. After 15 min recovery, norepinephrine was infused into the LAD perfusion line (0.05–0.75 μg·kg⁻¹·min⁻¹). Each norepinephrine infusion rate was maintained for ~3 min, and data were recorded when coronary blood flow was stable at each level.

### Statistical Analyses

Data are presented as means ± SE. Hemodynamic variables were recorded with Powerlab 3.1.4 data-acquisition software (ADInstruments). A three-way ANOVA was used to compare the effects of diabetes, α-adrenoceptor blockade, and exercise on coronary and systemic hemodynamics at rest and during exercise in the diabetic dogs. A two-way ANOVA was used to test for differences in norepinephrine-mediated coronary vasoconstriction between the normal and diabetic control and diabetic dogs. When significance was found with ANOVA (P < 0.05), a Student-Newman-Keuls multiple-comparison test was performed. Linear regression analysis was used to compare the slope of the relationship between coronary venous PO2 vs. MV02. Regression analyses were performed on mean values. If the slopes of the regression lines were not significantly different (P > 0.05), an analysis of covariance was employed to test for differences in elevation (intercept).

### RESULTS

Hemodynamic, blood gas, and metabolic variables at rest and during exercise are given in Table 1. Body weight was reduced from 26.3 ± 1.7 to 24.5 ± 1.6 kg after alloxan administration. In nondiabetic control dogs, exercise increased coronary conductance 2.1-fold, coronary blood flow 2.5-fold, MV02 3.1-fold, and heart rate 2.5-fold (Table 1). In diabetic dogs, exercise increased coronary conductance 1.8-fold, coronary blood flow 2.2-fold, MV02 2.7-fold, and heart rate 2.3-

### Additional Notes

- The study design and methodology ensure accurate and reliable data collection.
- The use of statistical analyses supports the validity of the findings.
- The study contributes to the understanding of the effects of diabetes on coronary blood flow and metabolism.
fold. Under baseline, resting conditions, α-adrenoceptor blockade with phentolamine did not significantly increase coronary blood flow or conductance in the nondiabetic or diabetic animals (Table 1). Although these variables tended to be higher after α-blockade, this finding indicates that augmented α-vasoconstriction is not the mechanism by which diabetes impairs the balance between coronary blood flow and myocardial metabolism under baseline conditions.

A sensitive index of the relationship between coronary blood flow and myocardial metabolism is shown in the plot of coronary venous Po2 vs. MV02 (Fig. 1). MV02 is computed from the product of coronary blood flow and the arterial-coronary venous difference in oxygen content. Thus the illustrated variables are not completely independent. However, such a plot is crucial to coronary flow studies because MV02 is the primary determinant of coronary blood flow. Diabetes lowered coronary venous Po2 at a given MV02 (parallel shift, 0.02), indicating that diabetes impairs the balance between coronary blood flow and myocardial metabolism equally at rest and during exercise. This finding is consistent with earlier reports from our laboratory (47).

The percent increase in coronary blood flow (Fig. 2A) and coronary conductance (Fig. 2B) at rest and during exercise in nondiabetic and diabetic α-adrenoceptor blocked dogs is

| Table 1. Hemodynamic variables at rest and during graded treadmill exercise |
|---------------------------------|-----------------|-----------------|-----------------|
| Coronary conductance, μL/min−1·g−1·mmHg−1 | Rest | Level 1 | Level 2 | Level 3 |
| Control (n = 8) | 7.22±0.56 | 10.27±0.97 | 12.84±1.33* | 15.13±1.52* |
| Control + phentolamine (n = 6) | 10.91±1.70 | 20.49±4.18† | 22.07±4.81†† | 23.30±5.41†† |
| Diabetes (n = 8) | 7.33±0.73 | 9.09±1.07 | 11.11±0.88 | 13.04±1.33* |
| Diabetes + phentolamine (n = 6) | 9.67±1.87 | 17.80±4.03‡ | 22.94±3.77‡‡ | 23.84±2.94‡‡ |

Coronary blood flow, ml/min−1·g−1

Control (n = 8) | 0.70±0.04 | 1.19±0.11* | 1.39±0.10* | 1.77±0.14* |
| Control + phentolamine (n = 6) | 0.92±0.11 | 1.69±0.25† | 1.89±0.29†† | 2.06±0.36* |
| Diabetes (n = 8) | 0.67±0.03 | 0.90±0.06 | 1.20±0.09* | 1.45±0.15* |
| Diabetes + phentolamine (n = 6) | 0.75±0.07 | 1.55±0.23‡ | 1.83±0.19‡‡ | 2.02±0.17‡‡ |

Myocardial O2 consumption, μL O2·min−1·g−1

Control (n = 8) | 92±6 | 196±20* | 227±18* | 286±19* |
| Control + phentolamine (n = 6) | 116±16 | 219±38* | 252±44* | 285±60* |
| Diabetes (n = 8) | 91±8 | 139±13 | 193±21* | 244±32* |
| Diabetes + phentolamine (n = 6) | 96±7 | 211±29‡ | 252±26* | 295±26* |

Mean aortic pressure, mmHg

Control (n = 8) | 99±3 | 117±6* | 112±6* | 116±4* |
| Control + phentolamine (n = 6) | 89±5 | 88±7† | 87±5† | 89±6† |
| Diabetes (n = 8) | 95±5 | 103±5 | 108±4* | 111±3* |
| Diabetes + phentolamine (n = 6) | 79±7‡ | 85±5‡ | 79±7‡ | 83±8‡ |

Heart rate, beats/min

Control (n = 8) | 88±7 | 133±11* | 174±19* | 221±18* |
| Control + phentolamine (n = 6) | 161±23† | 221±30†† | 230±28†† | 240±23* |
| Diabetes (n = 8) | 97±10 | 139±10* | 178±10* | 219±15* |
| Diabetes + phentolamine (n = 6) | 115±21§ | 210±25‡‡ | 229±19‡‡ | 240±15‡ |

Arterial pH

Control (n = 8) | 7.43±0.02 | 7.45±0.02 | 7.46±0.02 | 7.45±0.01 |
| Control + phentolamine (n = 6) | 7.43±0.01 | 7.45±0.02 | 7.46±0.01 | 7.44±0.01 |
| Diabetes (n = 8) | 7.40±0.02 | 7.42±0.02 | 7.41±0.02 | 7.41±0.02 |
| Diabetes + phentolamine (n = 6) | 7.38±0.02§ | 7.40±0.02§ | 7.39±0.01§ | 7.39±0.02§ |

Arterial Po2, Torr

Control (n = 8) | 86±3 | 93±3 | 89±2 | 81±3 |
| Control + phentolamine (n = 6) | 80±3 | 84±2 | 85±2 | 83±2 |
| Diabetes (n = 8) | 86±3 | 90±2 | 86±3 | 85±2 |
| Diabetes + phentolamine (n = 6) | 86±3 | 91±2 | 90±4 | 88±3 |

Coronary venous Po2, Torr

Control (n = 8) | 18.8±0.7 | 14.3±0.7* | 14.9±0.5* | 14.5±0.6* |
| Control + phentolamine (n = 6) | 17.6±0.8 | 16.5±1.1 | 16.2±1.1 | 16.5±1.1 |
| Diabetes (n = 8) | 16.8±0.9 | 14.3±0.9* | 13.4±0.7* | 13.8±0.8* |
| Diabetes + phentolamine (n = 6) | 15.1±1.1§ | 15.3±0.9 | 15.0±0.8 | 15.4±0.7 |

Hematocrit, %

Control (n = 8) | 38±2 | 41±2* | 42±2* | 42±2* |
| Control + phentolamine (n = 6) | 37±1 | 40±2 | 39±2 | 39±1 |
| Diabetes (n = 8) | 40±2 | 41±1 | 42±1 | 44±2* |
| Diabetes + phentolamine (n = 6) | 36±0‡ | 37±1‡ | 38±1‡ | 39±1‡ |

Arterial plasma glucose concentration, mM

Control (n = 8) | 4.7±0.2 | 4.7±0.2 | 4.6±0.2 | 5.1±0.3 |
| Control + phentolamine (n = 6) | 4.5±0.3 | 4.4±0.3 | 4.6±0.2 | 4.7±0.2 |
| Diabetes (n = 8) | 21.4±1.9 | 20.9±1.9 | 21.1±2.0 | 20.7±1.9 |
| Diabetes + phentolamine (n = 6) | 16.4±1.2‡§ | 16.6±1.2‡§ | 16.6±0.7‡§ | 16.9±0.7‡§ |

Values are means ± SE; n, no. of dogs. *P < 0.01 vs. Rest same condition; †P < 0.01 vs. Control same condition; ††P < 0.01 vs. Diabetes same condition; §P < 0.05 vs. Control + phentolamine same condition.
shown in Fig. 2. The increase in both coronary blood flow and conductance to exercise was greater in the diabetic dogs relative to nondiabetic dogs, indicating that diabetes increases \( \alpha \)-adrenoceptor-mediated coronary vasoconstriction during increases in \( \dot{MVO}_2 \). Figure 3 shows the relationship between coronary venous \( \text{PO}_2 \) vs. \( \dot{MVO}_2 \) in nondiabetic control dogs (A) and diabetic dogs (B) before and after \( \alpha \)-adrenoceptor blockade with phentolamine. Phentolamine decreased the slope of this relationship, i.e., made the slope less negative, in both experimental groups, indicating that \( \alpha \)-adrenoceptor-mediated vasoconstriction limits coronary dilation during exercise in both nondiabetic and diabetic dogs. The difference between the untreated slope and the phentolamine-treated slope was greater in the diabetic dogs (control = \(-0.02 \text{Torr/\mu L min}^{-1} \text{g}^{-1}\) vs. phentolamine = 0.0009 Torr/\( \mu \text{L min}^{-1} \text{g}^{-1}\)) than in the nondiabetic control dogs (control = \(-0.02 \text{Torr/\mu L min}^{-1} \text{g}^{-1}\) vs. phentolamine = \(-0.0088 \text{Torr/\mu L min}^{-1} \text{g}^{-1}\)). In addition, the slope of the coronary venous \( \text{PO}_2 \) vs. \( \dot{MVO}_2 \) relationship after phentolamine treatment was significantly lower in the diabetic dogs. This difference in slope is supported by data presented in Fig. 4 that demonstrate that the change in coronary venous \( \text{PO}_2 \), relative to the resting-baseline condition (\%baseline), is significantly lower when \( \dot{MVO}_2 \) is elevated during exercise in the phentolamine-treated diabetic dogs than in the nondiabetic control dogs treated with phentolamine. This finding indicates that blockade of \( \alpha \)-adrenoceptors in diabetic dogs results in a more improved balance between coronary flow and metabolism when myocardial oxygen demand is elevated. Taken together, these results indicate that \( \alpha \)-adrenoceptor-mediated coronary vasoconstriction is augmented in alloxan-induced diabetic dogs during exercise-induced increases in \( \dot{MVO}_2 \).

Figure 5 shows the effects of graded norepinephrine infusion on coronary blood flow (\% baseline) in nondiabetic control and diabetic dogs. Propranolol (2 mg/kg iv) was administered to these animals to inhibit the \( \beta \)-adrenoceptor effects of norepinephrine, i.e., positive inotropic, chronotropic, and “feedforward” vasodilatation (16, 20, 35). Coronary perfusion pressure was maintained at 100 mmHg throughout the experimental protocol so that alterations in perfusion pressure would not influence the coronary flow response to norepinephrine. We found that the decrease in coronary blood flow to norepinephrine was significantly augmented at the lower doses of norepinephrine in the diabetic dogs compared with the nondiabetic dogs.

**DISCUSSION**

**\( \alpha \)-Adrenoceptor Coronary Vasoconstriction in Diabetes**

The purpose of the present investigation was to examine the hypothesis that \( \alpha \)-adrenoceptor-mediated coronary vasoconstriction is augmented during physiological increases in myocardial oxygen demand in experimental type I diabetes. We found that the increase in coronary blood flow and conductance to exercise was greater after \( \alpha \)-adrenoceptor blockade in the diabetic dogs (Fig. 2) and that the difference in the slope of the coronary venous \( \text{PO}_2 \) vs. \( \dot{MVO}_2 \) relationship between the untreated control and phentolamine-treated groups was greater in the diabetic dogs than in the normal, control dogs (Fig. 3). In addition, the slope of the coronary venous \( \text{PO}_2 \) vs. \( \dot{MVO}_2 \) relationship after phentolamine treatment was significantly lower in the diabetic dogs compared with the nondiabetic control dogs; i.e., the decrease in coronary venous \( \text{PO}_2 \) as a
function of MVO₂ was diminished in the diabetic dogs (Fig. 4). These results indicate that α-adrenoceptor-mediated coronary vasoconstriction is augmented in alloxan-induced diabetic dogs during exercise-induced increases in MVO₂. The decrease in coronary venous PO₂ was significantly diminished in the diabetic dogs, suggesting that α-adrenoceptor-mediated coronary vasoconstriction is augmented during exercise relative to nondiabetic controls.

To further test the hypothesis that α-adrenoceptor-mediated coronary vasoconstriction is augmented in diabetic subjects, additional experiments were performed in anesthetized, open-chest, β-adrenoceptor blocked dogs. In these studies, we found that coronary vasoconstriction to the lower, more physiological doses of norepinephrine was significantly increased in the diabetic dogs (Fig. 5). This supports our findings from the conscious, exercise studies and indicates that experimental type I diabetes augments α-adrenoceptor-mediated coronary vasoconstriction. Earlier studies have reported that diabetes increases myocardial α-adrenoceptor sensitivity but decreases the overall number of α-adrenoceptor binding sites (27, 32, 48). However, whether α-adrenoceptor density or sensitivity of coronary vascular smooth muscle cells is altered by diabetes is presently unknown.

Although α-adrenoceptor-mediated coronary vasoconstriction restricts increases in coronary blood flow, it has been shown to be important in maintaining blood flow to the vulnerable subendocardium when heart rate, contractility, and MVO₂ are elevated during exercise (16, 29, 45). The postulated
mechanism is that increased release of norepinephrine during exercise stimulates α-adrenoceptor vasoconstriction of medium-sized intramyocardial vessels (diameter > 100 μm), which decreases intramyocardial vascular capacitance and wasteful antegrade-retrograde flow oscillations during the cardiac cycle (36). This hypothesis is supported by findings indicating that α₁-adrenoceptor-mediated vasoconstriction occurs predominantly in larger coronary vessels (diameter > 100 μm) (7, 8, 26) and that the limitation of exercise-induced increases in coronary blood flow is primarily due to α₁-adrenoceptor vasoconstriction (3, 4, 10, 12, 23). However, it should be pointed out that other investigations have shown that α-adrenoceptor-mediated coronary vasoconstriction does not affect transmural blood flow distribution (5, 12). The reason for this discrepancy is unclear, but it may be related to the use of different α-adrenoceptor antagonists, i.e., phentolamine vs. phenoxybenzamine. In addition, studies indicate that α₂-adrenoceptors contribute to coronary vasoconstriction during sympathetic stimulation (26) and during ischemia (25, 30, 39). Whether the augmentation of α-adrenoceptor coronary vasoconstriction in diabetes is mediated by α₁ and/or α₂-adrenoceptors merits future investigation. Additional studies are also needed to determine whether this increase in α-vasoconstriction alters transmural blood flow distribution during increases in MV₂O₂.

Confounding Effects of α-Adrenoceptor Blockade

Although the findings of this investigation indicate that α-adrenoceptor-mediated coronary vasoconstriction is augmented in alloxan-induced diabetic dogs during exercise, it must be acknowledged that mechanisms other than abolition of α-adrenoceptor-mediated vasoconstrictor tone could be activated to cause coronary vasodilation after phentolamine administration. Systemic administration of phentolamine, as performed in the present investigation, increases circulating and cardiac norepinephrine concentrations, which may increase feedforward β-adrenoceptor vasodilation (16, 20, 21, 35). Administering a β-adrenoceptor antagonist during exercise would not quantify this confounding effect of phentolamine because any decrease in the balance between coronary blood flow and myocardial metabolism (i.e., decrease in slope of coronary venous PO₂ vs. MV₂O₂) after β-blockade could be due to either the loss of normal β-adrenoceptor vasodilation, loss of augmented β-adrenoceptor vasodilation, or a combination of the two. Because these experiments cannot differentiate between normal and augmented β-adrenoceptor-mediated coronary vasodilation, they would not help quantify the augmentation effect. In addition, a similar problem exists if β-adrenoceptor blockade alone is used because elevated catecholamine release would exaggerate the unopposed α-adrenoceptor vasoconstriction (41). Therefore, quantifying the degree of augmented β-adrenoceptor-mediated vasodilation after α-adrenoceptor blockade during exercise is not possible.

To address this question, we conducted experiments in anesthetized, open-chest dogs in which the LAD was cannulated and perfused by a extracorporeal perfusion system so that graded doses of norepinephrine could be infused into the coronary circulation while coronary perfusion pressure was maintained constant. These experiments were conducted in nondiabetic and alloxan-induced diabetic dogs in the presence of propranolol to inhibit β-adrenoceptor-mediated increases in heart rate, contractility, and β-feedforward coronary vasodilation. We found that the decrease in coronary blood flow to lower doses norepinephrine was greater in the diabetic dogs relative to the nondiabetic control dogs (Fig. 5). This finding further supports the hypothesis that α-adrenoceptor-mediated coronary vasoconstriction is increased in experimental diabetes and argues against a significant augmentation of β-adrenoceptor-mediated vasodilation after phentolamine in the exercise studies. In other words, if the present findings were due entirely to enhanced β-adrenoceptor-mediated effects then there would have been no change in norepinephrine-induced coronary vasoconstriction in the diabetic dogs. A further argument against an increase in β-adrenoceptor vasodilation is that β-adrenoceptor density (27, 32) is significantly reduced in streptozocin-induced diabetic rats with little or no change in receptor affinity (2, 27).

A recent study by Takamura et al. (42) found that nitric oxide-mediated vasodilation was enhanced after α-adrenoceptor inhibition in exercising dogs. This finding is intriguing in that it suggests that after α-adrenoceptor inhibition there is a significant increase in to-and-fro flow oscillation, i.e., shear stress (36), which stimulates an increase in nitric oxide production. Such a mechanism could have contributed to the greater increases in coronary blood flow and conductance in the diabetic animals, if nitric oxide release in the diabetic dogs was greater than that of the nondiabetic control dogs. Although several studies have reported that endothelium-dependent vasodilation is significantly impaired in experimental diabetic animals (17, 19, 34, 37, 49), others have reported an increase in endothelium-dependent relaxation (9, 38). Thus, whether nitric oxide-mediated vasodilation is enhanced after α-adrenoceptor inhibition in diabetic dogs merits further investigation.

Endothelin and α-Adrenoceptor Coronary Vasosconstriction

Although endothelium-dependent vasodilation may be depressed in diabetics (17, 19, 34, 37, 49), it is doubtful that a decrease in nitric oxide dilation is solely responsible for the coronary dysfunction induced by diabetes because nitric oxide is not required for balancing coronary blood flow with myocardial metabolism during increases in MV₂O₂ (1, 6, 15, 44). However, endothelial dysfunction is characterized not only by decreased nitric oxide production and/or vasodilation but also by increases in vasoconstricting factors such as endothelin (33). Recent studies have reported increases in circulating endothelin levels in diabetic rats (28) and humans (13). Interestingly, increased circulating endothelin levels in diabetic subjects could be linked with elevated α-adrenoceptor activation because α-adrenergic stimulation of cardiac myocytes results in endothelin-dependent coronary vasoconstriction both in vitro (43) and in vivo (11). Whether augmented α-adrenoceptor stimulation increases endothelin-induced coronary vasoconstriction in diabetic subjects has not been investigated.

Limitations of the Study

A major limitation of the present investigation is the acute diabetic state and loss of body weight (~7%) in the alloxan-treated dogs. This model does not represent the current clinical course of long-term diabetes that elicits coronary disease. Thus future experiments will require study of augmented α-adreno-
ceptor-mediated coronary vasoconstriction in stable, chronically diabetic animals.

In conclusion, this study is the first to document that α-adrenoceptor-mediated coronary vasoconstriction is augmented in alloxan-induced diabetic dogs during physiological increases in $\dot{\text{V}}$O$_2$. This increase in sympathetic vasoconstriction is one mechanism by which diabetes decreases myocardial oxygen delivery and imbalances the pressure between coronary blood flow and myocardial metabolism during exercise (47).

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GRANTS

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