Autonomic Control of Vasomotion in the Porcine Coronary Circulation During Treadmill Exercise : Evidence for Feed-Forward ß-Adrenergic Control

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Autonomic Control of Vasomotion in the Porcine Coronary Circulation During Treadmill Exercise

Evidence for Feed-Forward β-Adrenergic Control

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Abstract—To date, no studies have investigated coronary vasomotor control of myocardial O_2 delivery (MDO_2) and its modulation by the autonomic nervous system in the porcine heart during treadmill exercise. We studied 8 chronically instrumented swine under resting conditions and during graded treadmill exercise. Exercise up to 85% to 90% of maximum heart rate produced an increase in myocardial O_2 consumption ($M\dot{V}O_2$) from 163±16 μmol/min (mean±SE) at rest to 423±75 μmol/min ($P \leq 0.05$), which was paralleled by an increase in MDO_2, so that myocardial O_2 extraction (79±1% at rest) and coronary venous O_2 tension ($cvPO_2$, 23.7±1.0 mm Hg at rest) were maintained. β-Adrenoceptor blockade blunted the exercise-induced increase of MDO_2 out of proportion compared with the attenuation of the exercise-induced increase in $M\dot{V}O_2$, so that O_2 extraction rose from 78±1% at rest to 83±1% during exercise and $cvPO_2$ fell from 23.5±0.9 to 19.6±1.1 mm Hg (both $P \leq 0.05$). In contrast, α-adrenoceptor blockade, either in the absence or presence of β-adrenoceptor blockade, had no effect on myocardial O_2 extraction or $cvPO_2$ at rest or during exercise. Muscarinic receptor blockade resulted in a decreased O_2 extraction and an increase in $cvPO_2$ at rest, an effect that waned during exercise. The vasodilation produced by muscarinic receptor blockade was likely due to an increased β-adrenoceptor activity, since combined muscarinic and β-adrenoceptor blockade produced similar changes in O_2 extraction and $cvPO_2$, as did β-adrenoceptor blockade alone. In conclusion, in swine myocardium, $M\dot{V}O_2$ and MDO_2 are matched during exercise, which is the result of feed-forward β-adrenergic vasodilation in conjunction with minimal α-adrenergic vasoconstriction. β-Adrenergic vasodilation is due to an increase in sympathetic activity but may also be supported by withdrawal of muscarinic receptor–mediated inhibition of β-adrenergic coronary vasodilation. The observation that $cvPO_2$ levels are maintained even during heavy exercise suggests that a decrease in $cvPO_2$ is not essential for coronary vasodilation during exercise. (***Circ Res*. 1998;82:1312-1322.)**

Key Words: coronary blood flow ■ exercise ■ myocardial O_2 extraction ■ autonomic nervous system ■ myocardial O_2 consumption

In the normal heart, coronary blood flow is tightly regulated in response to changing metabolic needs to maintain a consistently high level of myocardial O_2 extraction. The close coupling of coronary blood flow and myocardial O_2 demand has been proposed to depend primarily on messengers released from the myocardium and endothelium but is also modulated by the autononomic nervous system.[1,2] Thus, in dogs, the treadmill exercise–induced increases in coronary blood flow and, hence, O_2 delivery do not fully match the increase in myocardial O_2 demand, so that even at mild to moderate levels of exercise (<70% of maximum heart rate), myocardial O_2 extraction increases[3–7] and $cvPO_2$ decreases.[5,7–9] The decrease in $cvPO_2$ may represent a metabolic error signal needed for a negative-feedback control mechanism[1,10] but is at least in part due to α-adrenergic vasoconstriction of coronary resistance vessels.[7,9,11] Conversely, the increase in sympathetic nerve activity during exercise also results in β-adrenergic coronary vasodilation,[5,12] which acts in a "feed-forward control" manner (ie, an open-loop control system that does not require an error signal[13,14]) to blunt the α-adrenergic vasoconstriction in dogs.

To our knowledge, the only other mammalian heart in which the balance between MDO_2 and O_2 utilization has been studied during exercise is the human heart.[1,2] In humans, myocardial O_2 extraction,[15–20] $cvSO_2$,[16,18–20] cvO_2 content,[15,16,19–21] or $cvPO_2$[19,22] are, in contrast to dogs, minimally affected during mild to moderate exercise (<80% of maximum heart rate), but an increase in fractional O_2 extraction and a decrease in cvO_2 content occur during heavy exercise (>85% of maximum heart rate).[18,23–25] In humans, the minimal changes in $cvPO_2$ at low to moderate levels of exercise could be due to an increased importance of β-adrenergic coronary vasodilation[26,27] or decreased importance of α-adrenergic vasoconstriction, but this has not been studied in

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Selected Abbreviations and Acronyms

cvO_2 = coronary venous O_2
cvP_{CO_2} = coronary venous P_{CO_2}
cvpH = coronary venous pH
cvP_{O_2} = coronary venous O_2 tension
cvS_{O_2} = coronary venous S_{O_2}
f_0 = emitting frequency
Hb = hemoglobin
LAD = left anterior descending coronary artery
LV = left ventricle
$M\dot{V}_{O_2}$ = myocardial O_2 consumption
MD_{O_2} = myocardial O_2 delivery
PVC = polyvinylchloride
S_{O_2} = O_2 saturation

humans. However, these findings indicate that considerable interspecies differences of coronary vasomotor control may exist during exercise. Therefore, in the present study, we investigated coronary vasomotor control of myocardial O_2 balance during treadmill exercise in another mammalian species, ie, swine, and observed that fractional myocardial O_2 extraction and cvP_{O_2} did not change even during heavy treadmill exercise (85% to 90% of maximum heart rate). We hypothesized that this could be the result of (1) negligible α-adrenergic coronary vasoconstriction in swine,[28] (2) increased importance of feed-forward β-adrenergic vasodilation,[12–14] or (3) withdrawal of vagal constrictor tone.[29–31] To determine the relative contributions of each of these potential mechanisms, we studied myocardial O_2 balance in swine during treadmill exercise in the absence and presence of single and combined blockade of α- and β-adrenergic receptors and muscarinic receptors.

Materials and Methods

Eight crossbred Landrace×Yorkshire pigs (5 male and 3 female) were used in the present study. All experiments were performed in accordance with the *Guiding Principles in the Care and Use of Laboratory Animals* as approved by the Council of the American Physiological Society and with the prior approval of the Animal Care Committee of the Erasmus University Rotterdam. Adaptation of animals to the laboratory conditions started 1 week before the day of surgery and continued until 10 days after surgery. Full details of the experimental procedures have been published previously.[32–34]

Surgical Procedures

After an overnight fast, pigs (23±1 kg) were sedated with 30 mg/kg IM ketamine (Ketalin, Apharmo BV), anesthetized with thiopental (10 mg/kg IV, Nesdonal, Rhône-Poulenc Rorer BV), intubated, and mechanically ventilated with a mixture of O_2 and nitrous oxide (1:2), to which 0.2% to 1% (vol/vol) isoflurane (Forene, Abbott BV) was added. Anesthesia was further maintained with midazolam (2 $mg/kg+1\ mg \cdot kg^{-1} \cdot h^{-1}$ IV, Dormicum, Roche BV) and fentanyl ($10\ \mu g \cdot kg^{-1} \cdot h^{-1}$ IV, Fentanyl-Janssen, Janssen-Cilag BV). Under sterile conditions, the chest was opened via the fourth left intercostal space, and an 8F fluid-filled PVC catheter was inserted into the aortic arch (for the measurement of central aortic blood pressure and collection of arterial blood samples) and secured with a purse-string suture. After the pericardium was opened, a high-fidelity pressure transducer (model $P_{4.5}$, Konigsberg Instruments Inc) was inserted into the LV via the apical dimple for recording of LV pressure and its first derivative (LV dP/dt, obtained via electrical differentiation). An 8F PVC catheter was also inserted into the LV for calibration of the Konigsberg transducer signal; another 8F PVC catheter was inserted into the pulmonary artery for administration of drugs. A Doppler flow probe (inner diameter, 2.0 to 3.0 mm; f_0, 20 MHz) was placed around the proximal part of the LAD to measure the coronary Doppler shift. A small angiocatheter (inner/outer diameter, 0.8 mm/1.1 mm) connected to a larger Tygon catheter (inner/outer diameter, 0.8 mm/2.4 mm) was inserted directly into the anterior interventricular vein to allow sampling of coronary venous blood.[35] Electrical wires and catheters were tunneled subcutaneously to the back, the chest was closed, and the animals were allowed to recover. All electrical wires and catheters were protected with a vest.

Postsurgical Period

During the first week after surgery animals received intravenous injections of 25 mg/kg amoxicillin (Clamoxil, Beecham Farma BV) and 5 mg/kg gentamicin (Ad Usum Veterinarium) on a daily basis to prevent infection. Catheters were flushed daily with physiological saline containing 2000 IU/mL heparin.

Experimental Protocols

Studies were performed 10 to 20 days after surgery with animals exercising on a motor-driven treadmill. With swine lying quietly on the treadmill, resting hemodynamic measurements, consisting of LV pressure, LV dP/dt, aortic blood pressure, and the coronary Doppler shift, were obtained, and arterial and coronary venous blood samples were collected. In 2 of the 8 animals, samples could not be obtained from the coronary venous catheter. Hemodynamic measurements were repeated, and rectal temperature was measured with animals standing on the treadmill. Subsequently, a 5-stage treadmill exercise protocol was started (1, 2, 3, 4, and 5 km/h); each exercise stage lasted 2 to 3 minutes. LV pressure, LV dP/dt, aortic blood pressure, and coronary blood flow were continuously measured, and blood samples were collected during the last 30 seconds of each exercise stage, when hemodynamics had reached a steady state. After completing the exercise protocol, animals were allowed to rest on the treadmill.

After 90 minutes of rest, animals underwent 1 of 4 protocols, which were performed in random order and with each protocol performed on a different day. In protocol 1, we studied the reproducibility of 2 consecutive exercise periods. For this purpose, animals received (via the pulmonary artery catheter) an intravenous infusion of saline (10 mL) at a rate of 2 mL/min; 5 minutes after the infusion, resting measurements were obtained, and the 5-stage exercise protocol was repeated. In protocol 2, we studied α-adrenergic vasomotor control of the coronary circulation during exercise. For this purpose, animals received an intravenous infusion of phentolamine (1 mg/kg in 10 mL saline, administered over 5 minutes) to produce α-adrenergic receptor blockade. We have previously shown that this dose of phentolamine results in >95% inhibition of the intra-arterial noradrenaline (0.3 μg/kg)–induced increase in carotid vascular resistance in anesthetized swine.[36] Five minutes after completion of the infusion, resting measurements were obtained, and the exercise protocol was repeated. In protocol 3, we studied β-adrenergic vasomotor control of the coronary circulation during treadmill exercise and studied the α-adrenergic vasomotor control of the coronary circulation in the presence of β-adrenergic receptor blockade. For this purpose, animals received an intravenous infusion of propranolol (0.5 mg/kg, dissolved in 10 mL saline administered over 5 minutes) to produce β-adrenergic receptor blockade. This dosage regimen results in >95% inhibition of isoproterenol-induced increases in heart rate and LV dP/dt_{max} in awake swine.[32] Five minutes later, the exercise protocol was repeated. After another 90 minutes of rest, propranolol (0.2 mg/kg IV, in 4 mL saline) was readministered. We have previously shown that this dosage regimen of propranolol produces identical responses during 2 consecutive exercise protocols.[34] Five minutes later, α-adrenergic receptor blockade was produced by infusing phentolamine in a dose of 1 mg/kg IV (in 10 mL of saline) at a rate of 2 mL/min, and the exercise protocol was repeated. In protocol 4, we studied muscarinic control of the coronary circulation and studied β-adrenergic vasomotor control of the coronary circulation in the presence of muscarinic receptor blockade. For this purpose, animals received an

intravenous infusion of atropine (30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ at a rate of 1 mL/min) to produce muscarinic receptor blockade. This dose was considered to produce complete vagal blockade, because higher doses did not produce further increases in heart rate. Ten minutes after the start of the infusion, when hemodynamics had reached a new steady state, resting measurements were obtained, and the exercise protocol was repeated. After completion of the exercise protocol, the atropine infusion was interrupted, and animals were allowed to rest for another 90 minutes. Then, animals received an intravenous infusion of propranolol (0.5 mg/kg, dissolved in 10 mL saline) administered at a rate of 2 mL/min. After completion of propranolol administration, the infusion of atropine (30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ IV) was restarted, and 10 minutes later, the exercise protocol was repeated.

Hemodynamic Measurements

Aortic blood pressure was measured using a Combitrans pressure transducer (Braun) with the reference point at midchest level. LV pressure was measured with the Konigsberg micromanometer, which was calibrated with the fluid-filled LV catheter. The coronary Doppler shift was measured with a high-velocity pulsed Doppler velocimeter (model HVPD-20, Crystal Biotech).

Blood Gas Measurements

Blood samples were maintained in iced syringes until the conclusion of each exercise trial. Measurements of P_{O_2} (mm Hg), P_{CO_2} (mm Hg), and pH were then immediately performed with a blood gas analyzer (Acid-Base Laboratory model 505, Radiometer). S_{O_2} and Hb (g/100 mL) were measured with a hemoximeter (OSM2, Radiometer). Blood O_2 content (μmol/mL) was computed as follows: $(Hb \times 0.621 \times S_{O_2}) + (0.00131 \times P_{O_2})$. MD_{O_2} was computed as the product of LAD coronary blood flow and arterial blood O_2 content; $M\dot{V}_{O_2}$ in the region of myocardium perfused by the LAD was calculated as the product of coronary blood flow and the difference in O_2 content between arterial and coronary venous blood. Myocardial O_2 extraction was computed as the ratio of the arteriovenous O_2 content difference and the arterial O_2 content.

Data Acquisition and Analysis

Hemodynamic data were recorded and digitized on-line using an 8-channel data-acquisition program (ATCODAS, Dataq Instruments Inc) and stored on a computer for later postacquisition off-line analysis with a program written in MatLab (The Mathworks Inc). A minimum of 15 consecutive beats was selected for analysis of the digitized hemodynamic signals. From these selected beats, the LV peak systolic and LV end-diastolic blood pressure, mean aortic blood pressure, and mean coronary Doppler shift were determined for each beat and averaged.

Mean coronary blood flow was computed from the mean Doppler shift using the following equation: $Q = 1.25 \cdot \Delta f \cdot d^2$, where Q is the coronary blood flow (mL/min), Δf is the Doppler shift (kHz), and d is the internal diameter of the coronary artery (mm) within the flow probe.[37] The factor 1.25 is a constant derived from the speed of sound in tissue ($c = 1.5 \times 10^5$ cm/s), the frequency of the emitted sound beam ($f_0 = 20$ MHz), the cosine of the angle at which the sound beam is emitted (45°), and unit conversion factors: $(c \times 0.75\pi)/(2f_0 \times \cos 45^\circ)$.[37] Since in chronically instrumented animals the flow probe is tightly adhered to the coronary artery, the internal diameter of the flow probe is equal to the external diameter of the artery. To obtain the inner diameter of the coronary artery, we subtracted 10% of the external diameter of the coronary artery, which is approximately the arterial wall thickness. In this way, any error in computation of the coronary internal diameter would affect control and intervention conditions equally. Coronary vascular resistance was computed as the ratio of mean aortic pressure and coronary blood flow.

Statistical analysis was performed using 2-way (exercise and treatment) ANOVA for repeated measures. When a significant effect of exercise was observed, post hoc testing was performed using the Dunnett test. When a significant effect of treatment was observed, post hoc testing was performed using the Student-Newman-Keuls test. A value of $P \leq 0.05$ was considered statistically significant (2-tailed). All data are presented as mean±SE.

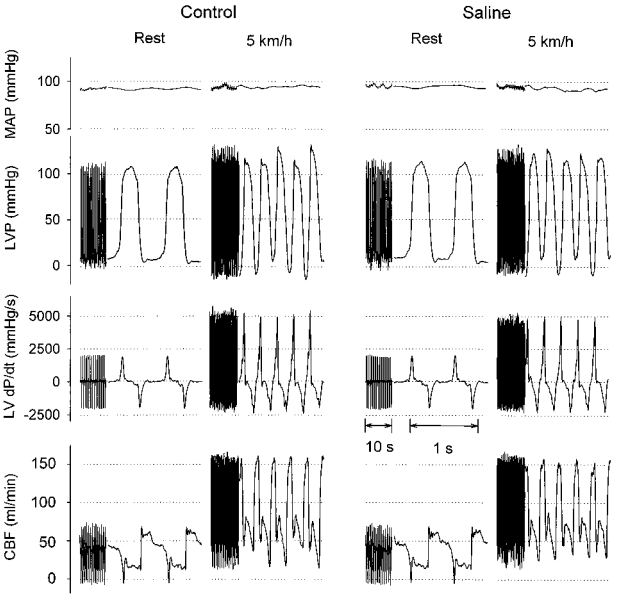

Figure 1. Recordings of hemodynamic data in an individual animal at rest (lying) and during exercise (5 km/h) during control conditions and in the presence of saline. MAP indicates mean aortic pressure; LVP, LV pressure; LV dP/dt, first derivative of LVP; and CBF, blood flow in the LAD.

Drugs

Phentolamine (10 mg/mL, Regitine, CIBA-Geigy BV) was dissolved in water containing glucose (35 mg/mL) and further diluted in saline to produce a final concentration of 1 $mg \cdot kg^{-1} \cdot 10\ mL^{-1}$. Propranolol (Sigma-Aldrich NV) was dissolved in 30°C saline to produce a concentration of 0.5 $mg \cdot kg^{-1} \cdot 10\ mL^{-1}$. Atropine (Sigma-Aldrich NV) was dissolved in 30°C saline to produce a concentration of 30 $\mu g \cdot kg^{-1} \cdot mL^{-1}$. Fresh drug solutions were prepared on each day.

Results

Reproducibility of Responses to Exercise

Hemodynamics

A representative example of the effects of saline on the hemodynamic data in an individual animal at rest and during exercise is shown in Figure 1; average data for all animals are presented in Figure 2. Exercise resulted in increases in heart rate (from 114±5 bpm at rest to 242±3 bpm at 5 km/h), LV systolic pressure (from 118±4 to 149±4 mm Hg), and LV dP/dt_{max} (from 3110±240 to 6600±380 mm Hg/s) (all $P \leq 0.05$) but had no significant effects on mean aortic pressure and LV end-diastolic pressure (8±2 mm Hg at rest). LAD blood flow increased from 40±3 mL/min at rest to 89±11 mL/min during exercise at 5 km/h. After 90 minutes of rest, at a time when all hemodynamic variables had returned to baseline resting values, the second period of exercise resulted in nearly identical hemodynamic responses to exercise.

Myocardial O_2 Balance

Exercise resulted in a slight decrease in arterial P_{CO_2} (from 44±2 mm Hg at rest to 40±2 mm Hg at 5 km/h) and an

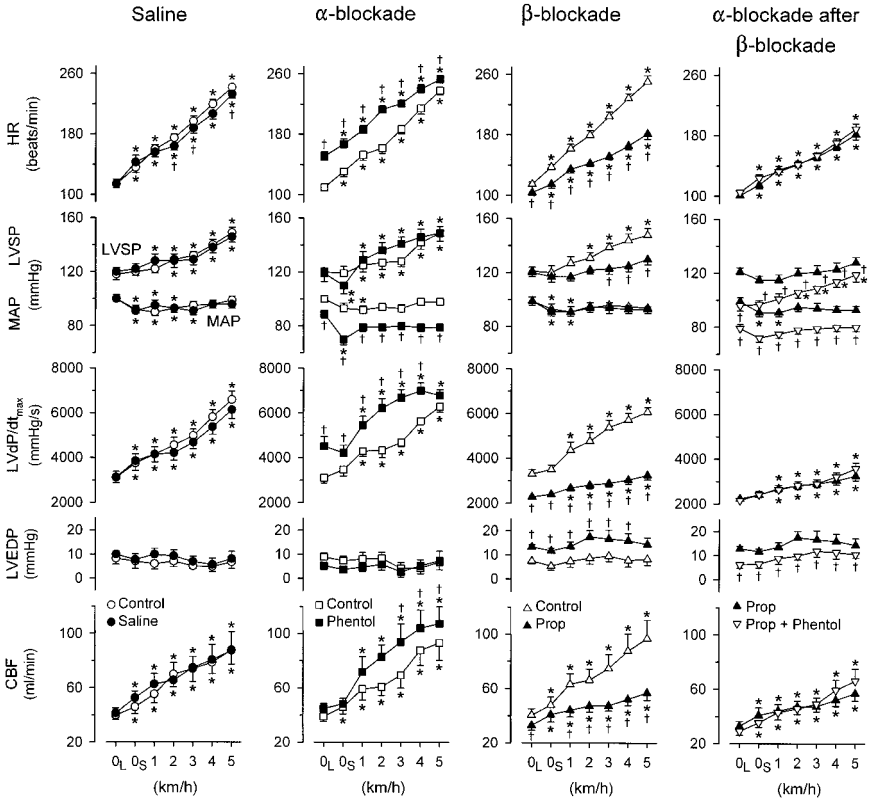

Figure 2. Systemic hemodynamic data at rest and during graded treadmill exercise. Shown are the effects of saline, α-adrenergic receptor blockade (α-blockade) produced by phentolamine (Phentol, 1 mg/kg IV), the effects of β-adrenergic receptor blockade (β-blockade) produced by propranolol (Prop, 0.5 mg/kg IV), and the effect of α-adrenergic blockade in the presence of β-adrenergic receptor blockade (α-blockade after β-blockade). Data points were obtained at rest (lying [0_L] and standing [0_S]) and during 5 levels of treadmill exercise (1 to 5 km/h). HR indicates heart rate; LVSP, LV systolic pressure; MAP, mean aortic pressure; LV dP/dt_{max}, maximum rate of rise of LVP; LVEDP, LV end-diastolic pressure; and CBF, coronary blood flow. Data are mean±SE (n=8). *$P \leq 0.05$ vs rest (lying); †$P \leq 0.05$ vs corresponding time point.

increase in pH (from 7.43±0.01 to 7.46±0.01) (both $P \leq 0.05$) but had no effect on arterial So_2 (96±1% at rest, lying, and during exercise at 5 km/h). Arterial Hb concentration (8.1±0.2 g% at rest and 9.1±0.4 g% at 5 km/h) and, hence, the arterial O_2 content (4.95±0.16 μmol/mL at rest and 5.54±0.20 μmol/mL at 5 km/h) increased by ≈12% at the highest level of exercise compared with resting conditions (both $P \leq 0.05$), whereas $cvPco_2$ (57±2 mm Hg at rest and 58±1 mm Hg at 5 km/h), cvpH (7.36±0.01 at rest and 7.37±0.01 at 5 km/h), $cvPo_2$ (23.7±1.0 mm Hg at rest and 23.4±1.3 mm Hg at 5 km/h), $cvSo_2$ (20.2±0.6% at rest and 20.1±1.2%), and cvO_2 content (1.04±0.06 μmol/mL at rest and 1.17±0.11 μmol/mL at 5 km/h) did not change from their respective resting values. $M\dot{V}o_2$ increased from 163±16 to 423±75 μmol/min, whereas MDo_2 delivery increased from 193±18 to 539±99 μmol/min (both $P \leq 0.05$), so that myocardial O_2 extraction (ie, the ratio between MDo_2 and $M\dot{V}o_2$) was not altered during exercise (Figure 3). All variables returned to baseline resting values within 90 minutes; a second period of exercise resulted in highly reproducible responses.

Effects of α-Adrenergic Receptor Blockade

Hemodynamics

Phentolamine produced a decrease in mean aortic pressure at rest and during treadmill exercise, which was accompanied by increases in heart rate and LV dP/dt_{max}, but no effect on LV

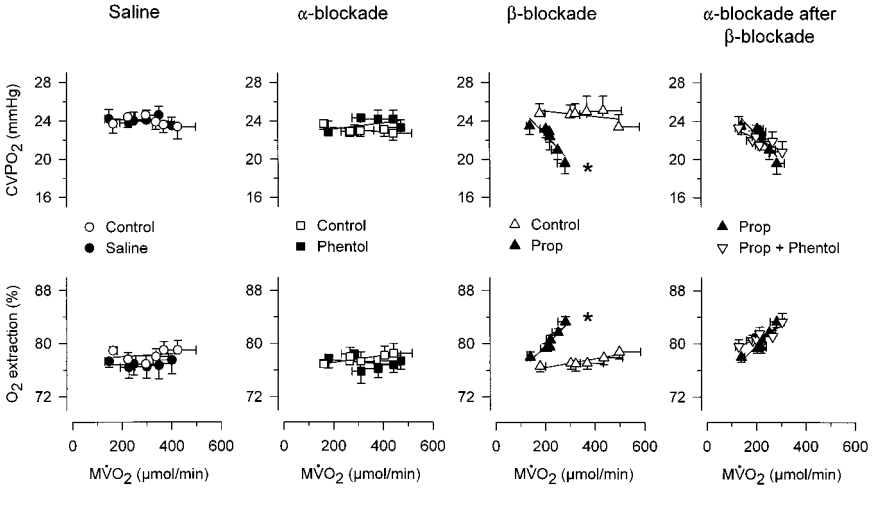

Figure 3. Relation between $M\dot{V}o_2$ and $cvPo_2$ (top panels) and between $M\dot{V}o_2$ and myocardial O_2 extraction (bottom panels). Shown are the effects of saline, α-adrenergic receptor blockade (α-blockade) produced by phentolamine (Phentol, 1 mg/kg IV), β-adrenergic receptor blockade (β-blockade) produced by propranolol (Prop, 0.5 mg/kg IV), and the effect of α-adrenergic blockade in the presence of β-adrenergic receptor blockade (α-blockade after β-blockade). Data points were obtained at rest (lying) and during 5 levels of treadmill exercise (1 to 5 km/h). Data are mean±SE (n=6). *$P \leq 0.05$.

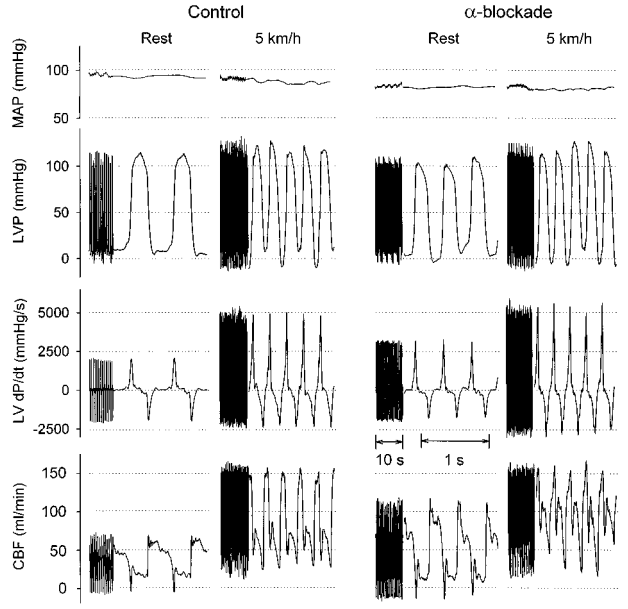

Figure 4. Recordings of hemodynamic data in an individual animal at rest (lying) and during exercise (5 km/h) during control conditions and in the presence of α-adrenergic receptor blockade (α-blockade) produced by phentolamine (1 mg/kg IV). MAP indicates mean aortic pressure; LVP, LV pressure; LV dP/dt, first derivative of LVP; and CBF, coronary blood flow.

peak systolic pressure or LV end-diastolic pressure (Figures 2 and 4). Coronary blood flow was not significantly altered under resting conditions, but at higher levels of exercise, coronary blood flow increased compared with control exercise.

Myocardial O_2 Balance

Phentolamine had no effect on arterial P_{CO_2}, pH, or S_{O_2} at rest or during exercise. In the presence of phentolamine arterial Hb concentration (8.3±0.3 g% at rest and 8.6±0.4 g% at 5 km/h, P=NS); hence, the arterial O_2 content no longer increased during exercise. Phentolamine had no significant effects on cvP_{CO_2}, cvpH, cvP_{O_2}, cvS_{O_2}, or cvO_2 content, either at rest or during exercise, except at 5 km/h, when pH was slightly reduced (7.34±0.01) compared with control exercise (7.38±0.01, $P \leq 0.05$). The relations between $M\dot{V}O_2$ and cvP_{O_2} or between $M\dot{V}O_2$ and O_2 extraction were not altered (Figure 3). However, because arterial O_2 content no longer increased during exercise, the relation between $M\dot{V}O_2$ and coronary blood flow shifted slightly upward toward higher blood flows compared with control exercise (not shown).

Effects of β-Adrenergic Receptor Blockade

Hemodynamics

Propranolol produced decreases in heart rate, LV dP/dt_{max}, and coronary blood flow and increases in LV end-diastolic pressure but had no effect on mean aortic pressure or LV systolic pressure in resting swine (Figures 2 and 5). Propranolol also markedly blunted the exercise-induced increases in heart rate, LV systolic pressure, LV dP/dt_{max}, and coronary blood flow compared with control exercise.

Myocardial O_2 Balance

Propranolol had no effect on arterial P_{CO_2}, pH, S_{O_2}, or Hb concentration; hence, there was no effect on the arterial O_2 content either at rest or during exercise. Similarly, cvP_{CO_2} and cvpH were also not altered by propranolol, but cvP_{O_2}, cvS_{O_2}, and cvO_2 content, which were not affected by propranolol during resting conditions, decreased progressively during exercise in the presence of propranolol (Figure 3). Consequently, the relation between $M\dot{V}O_2$ and cvP_{O_2} was shifted downward, and the relation between $M\dot{V}O_2$ and O_2 extraction was shifted upward.

Effects of α-Adrenergic Receptor Blockade in the Presence of β-Adrenergic Receptor Blockade

Hemodynamics

In the presence of propranolol, phentolamine produced decreases in mean aortic pressure and LV systolic pressure at rest and during treadmill exercise that were similar to the

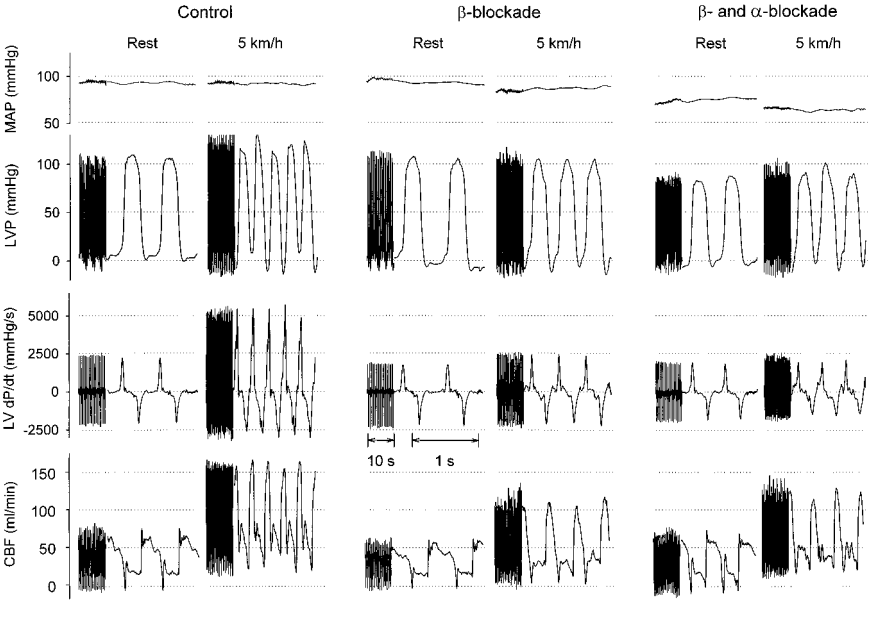

Figure 5. Recordings of hemodynamic data in an individual animal at rest (lying) and during exercise (5 km/h) during control conditions, in the presence of β-adrenergic receptor blockade (β-blockade) produced by propranolol (0.5 mg/kg IV), and in the presence of combined β-adrenergic and α-adrenergic receptor blockade (β- and α-blockade) produced by propranolol (0.2 mg/kg IV) and phentolamine (1 mg/kg IV). MAP indicates mean aortic pressure; LVP, LV pressure; LV dP/dt, first derivative of LVP; and CBF, coronary blood flow.

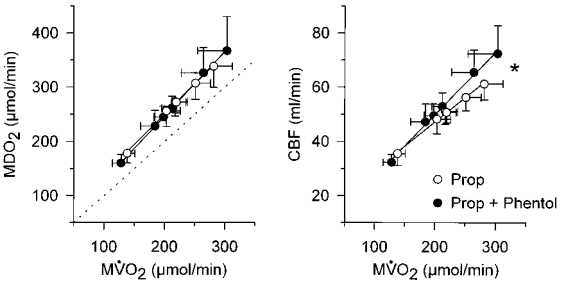

Figure 6. Relation between $M\dot{V}O_2$ and MDO_2 (left) and between $M\dot{V}O_2$ and coronary blood flow (CBF) (right). Shown are data points obtained at rest (lying) and during 5 levels of treadmill exercise in the presence of β-adrenergic receptor blockade produced by propranolol (Prop, 0.5 mg/kg IV) and in the presence of combined β-adrenergic and α-adrenergic receptor blockade produced by Prop (0.2 mg/kg IV) and phentolamine (Phentol, 1 mg/kg IV). Data are mean±SE (n=6). *$P \leq 0.05$.

effects of phentolamine in animals with intact β-adrenoceptors, but the increases in heart rate and LV dP/dt_{max} were abolished, whereas there was now a modest reduction in LV end-diastolic pressure (Figures 2 and 5). Phentolamine had no significant effect on coronary blood flow or coronary vascular resistance at rest or during exercise compared with propranolol alone.

Myocardial O_2 Balance

After propranolol, phentolamine had no effect on arterial PCO_2, pH, or SO_2 at rest or during exercise. Arterial Hb concentration and, hence, the arterial O_2 content failed to increase during exercise in the presence of phentolamine. $cvPCO_2$ and cvpH were not affected by phentolamine in the presence of propranolol, except at 5 km/h, when pH was slightly reduced (7.37±0.01) compared with propranolol treatment (7.39±0.01, $P \leq 0.05$). Phentolamine failed to increase $cvPO_2$, $cvSO_2$, or cvO_2 content and failed to produce changes in the relation between $M\dot{V}O_2$ and $cvPO_2$ or between $M\dot{V}O_2$ and O_2 extraction (Figure 3). Similarly, the relation between MDO_2 and $M\dot{V}O_2$ was not altered by phentolamine in the presence of propranolol (Figure 6). However, because arterial O_2 content failed to increase during exercise, the relation between $M\dot{V}O_2$ and coronary blood flow was shifted upward toward higher blood flows to compensate for the lower O_2 carrying capacity of the blood (Figure 6).

Effects of Muscarinic Receptor Blockade

Hemodynamics

Atropine produced increases in heart rate, LV systolic pressure, LV dP/dt_{max}, and coronary blood flow and decreases in LV end-diastolic pressure but had no effect on mean aortic pressure under resting conditions (Figures 7 and 8). The effects of atropine gradually waned at progressively higher levels of exercise.

Myocardial O_2 Balance

Atropine had no effect on arterial PCO_2, pH, SO_2, Hb concentration, or O_2 content either at rest or during exercise, except at 4 km/h, when PCO_2 was slightly higher (43±2 mm Hg) compared with control exercise (41±1 mm Hg, $P \leq 0.05$); $cvPCO_2$ and cvpH were also not affected by atropine. In contrast, $cvPO_2$, $cvSo_2$, and cvO_2 content increased significantly at rest and during exercise at 1 and 2 km/h, but these values were no longer different from control conditions at higher levels of exercise. Consequently, the relation between $M\dot{V}O_2$ and $cvPO_2$ shifted upward in the lower $M\dot{V}O_2$ range, whereas the relation between $M\dot{V}O_2$ and O_2 extraction shifted downward (Figure 9).

Effects of β-Adrenergic Receptor Blockade in the Presence of Muscarinic Receptor Blockade

Hemodynamics

The addition of β-adrenergic receptor blockade with propranolol to muscarinic receptor blockade with atropine resulted in decreases in heart rate, LV systolic pressure, LV dP/dt_{max}, and coronary blood flow at rest and during exercise and an increase in LV end-diastolic pressure but had no effect on mean aortic pressure (Figures 7 and 8).

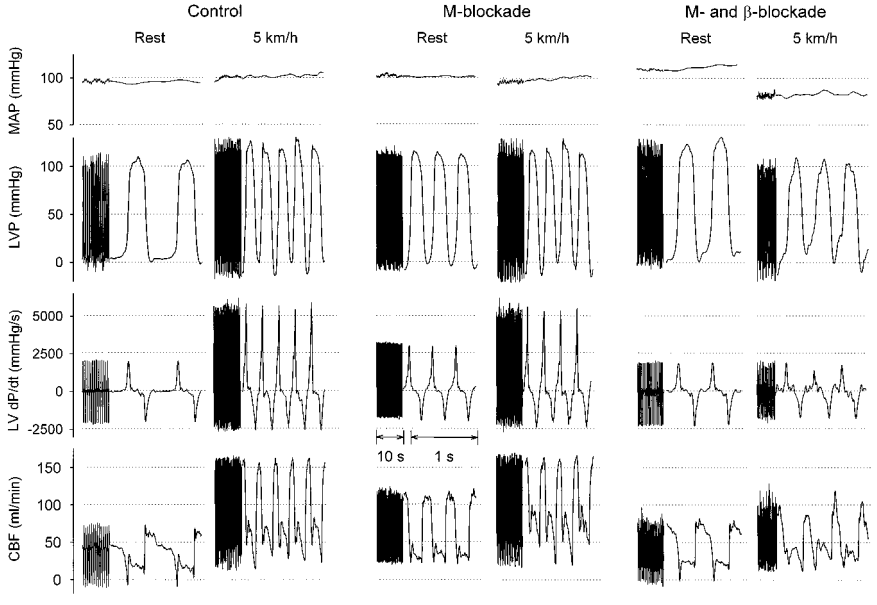

Figure 7. Recordings of hemodynamic data in an individual animal at rest (lying) and during exercise (5 km/h) during control conditions, in the presence of muscarinic receptor blockade (M-blockade) produced by atropine (30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ IV), and in the presence of combined muscarinic and β-adrenergic receptor blockade (M- and β-blockade) produced by atropine (30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ IV) and propranolol (0.5 mg/kg IV). MAP indicates mean aortic pressure; LVP, LV pressure; LV dP/dt, first derivative of LVP; and CBF, coronary blood flow.

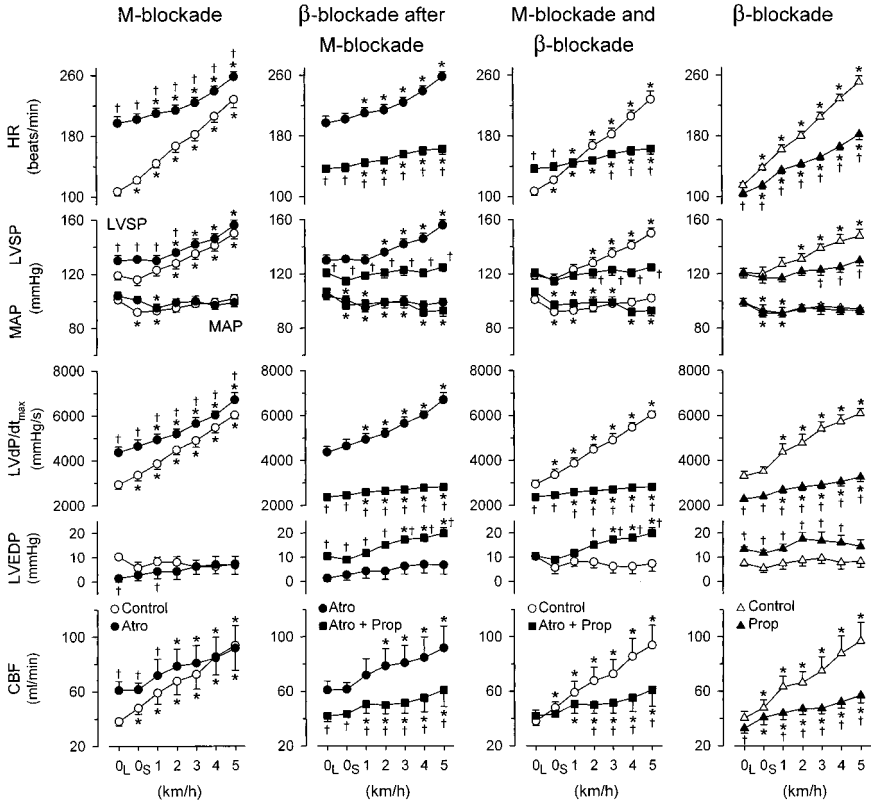

Figure 8. Systemic hemodynamic data at rest and during graded treadmill exercise. Shown are the effects of muscarinic receptor blockade (M-blockade) produced by atropine (Atro, 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ IV), the effects of β-adrenergic receptor blockade in the presence of muscarinic receptor blockade produced by propranolol (Prop, 0.5 mg/kg IV) in the presence of Atro (β-blockade after M-blockade), the effects of combined muscarinic and β-adrenergic receptor blockade produced by Atro and Prop (M-blockade and β-blockade), and the effects of β-adrenergic receptor blockade (β-blockade). Data points were obtained at rest (lying [0_L] and standing [0_S]) and during 5 levels of treadmill exercise (1 to 5 km/h). HR indicates heart rate; LVSP, LV systolic pressure; MAP, mean aortic pressure; LV dP/dt_{max}, maximum rate of rise of LVP; LVEDP, LV end-diastolic pressure; and CBF, coronary blood flow. Data are mean±SE (n=8). *$P \leq 0.05$ vs rest (lying); †$P \leq 0.05$ vs corresponding control or Atro measurement.

Myocardial O_2 Balance

The addition of propranolol to atropine had no effect on arterial P_{CO_2}, pH, S_{O_2}, Hb concentration, and O_2 content either at rest or during exercise. Similarly, cvP_{CO_2} and cvpH were also not affected by propranolol. cvP_{O_2}, cvS_{O_2}, and cvO_2 content were decreased both at rest and during exercise, so that the relation between $M\dot{V}_{O_2}$ and cvP_{O_2} was shifted downward, and the relation between $M\dot{V}_{O_2}$ and O_2 extraction was shifted upward (Figure 9). Importantly, the effects of combined atropine and propranolol on the relations between $M\dot{V}_{O_2}$ and cvP_{O_2} or O_2 extraction were not different from the effects of propranolol alone.

Discussion

The present study describes for the first time MD_{O_2} and extraction of the LV and its modulation by the autonomic nervous system in awake swine at rest and during treadmill exercise. The major findings of the present study were as follows: (1) in swine, myocardial O_2 extraction and cvP_{O_2} were not altered from resting levels during treadmill exercise at levels up to 85% to 90% of maximum heart rate; (2) α-adrenergic receptor blockade did not alter the relation between $M\dot{V}_{O_2}$ and myocardial O_2 extraction or the relation between $M\dot{V}_{O_2}$ and cvP_{O_2} (either in the absence or presence of β-adrenoceptor blockade), indicating that minimal α-ad-

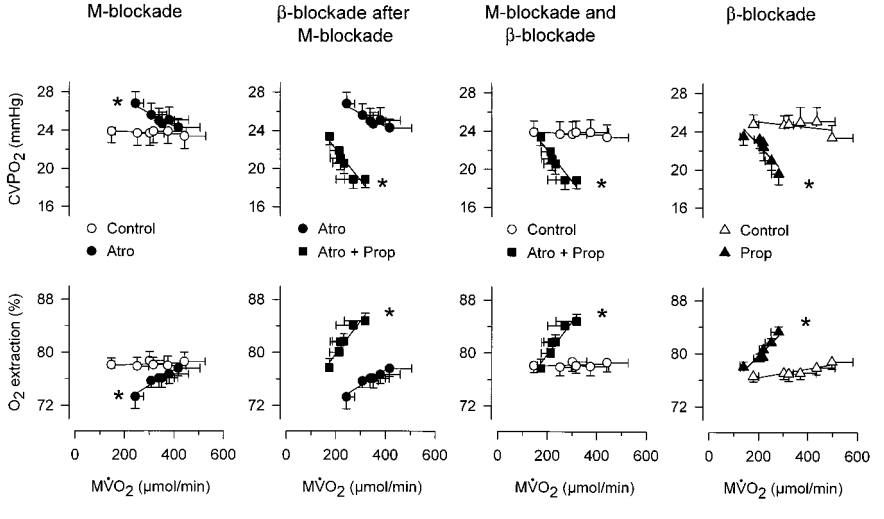

Figure 9. Relation between $M\dot{V}_{O_2}$ and cvP_{O_2} (top panels) and between $M\dot{V}_{O_2}$ and myocardial O_2 extraction (bottom panels). Shown are the effects of muscarinic receptor blockade (M-blockade) produced by atropine (Atro, 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ IV), β-adrenergic receptor blockade produced by propranolol (Prop, 0.5 mg/kg IV) in the presence of muscarinic receptor blockade (β-blockade after M-blockade), combined muscarinic and β-adrenergic receptor blockade (M-blockade and β-blockade), and β-adrenergic receptor blockade alone (β-blockade). Data points were obtained at rest (lying) and during 5 levels of treadmill exercise. Data are mean±SE (n=6). *$P \leq 0.05$.

renergic vasoconstriction occurs at rest and during exercise in swine; (3) β-adrenergic receptor blockade did not alter myocardial O_2 extraction or $cvPO_2$ under resting conditions but produced progressively greater myocardial O_2 extraction and produced a decrease in $cvPO_2$ during treadmill exercise, indicating that β-adrenoceptor activity was minimal under resting conditions but contributed in a feed-forward manner to coronary vasodilation during exercise; (4) muscarinic receptor blockade decreased myocardial O_2 extraction and increased $cvPO_2$ at rest and during mild exercise, but this effect disappeared at higher levels of exercise, indicating that muscarinic receptor activity exerted a vasoconstrictor influence on the coronary circulation only at rest and during low levels of exercise; and (5) the muscarinic vasoconstriction was most likely the result of inhibition of β-adrenergic vasodilator influence, since the effects of combined β-adrenergic receptor and muscarinic receptor blockade on the relation between $M\dot{V}O_2$ and either myocardial O_2 extraction or $cvPO_2$ were not different from the effects of β-adrenergic receptor blockade alone. The implications of these findings, which could not be explained by changes in arterial or coronary venous PCO_2 or pH, will be discussed in detail.

Myocardial O_2 Extraction During Treadmill Exercise

In dogs, the increase in coronary blood flow produced by treadmill exercise does not fully match the increased myocardial O_2 demand; thus, even during mild to moderate levels of exercise (<70% of maximum heart rate), an increase in O_2 extraction[3–7] and, hence, a decrease in $cvPO_2$ occur.[5,7–9] In contrast, in humans, minimal changes in O_2 extraction occur at mild to moderate levels of exercise,[15–20] although an increase in O_2 extraction and a decrease in cvO_2 content have been reported in humans during heavy exercise (>85% of maximum heart rate).[18,23–25] The present study indicates that at mild to moderate levels of exercise, swine resemble humans more closely than dogs. However, in contrast to dogs and humans, swine also maintain a constant level of myocardial O_2 extraction and $cvPO_2$ during heavy exercise, indicating that the exercise-induced increase in MDO_2 matches the increase in O_2 consumption. A decrease in $cvPO_2$ has been proposed to represent a metabolic error signal needed for a negative-feedback metabolic control mechanism,[1,10] which is necessary for the increase in coronary blood flow during exercise. The findings in the present study indicate that a decrease in $cvPO_2$ is not essential for the increase in coronary blood flow in the normal heart during heavy treadmill exercise.

Although swine lack a native collateral circulation, it could be argued that the instrumentation of a coronary artery may have resulted in collateralization of the myocardium perfused by the instrumented LAD. The collateral vessels could potentially supply the LAD bed with additional blood, thereby contributing to a maintained $cvPO_2$. Messina et al[38] demonstrated that significant collateral blood flow does not occur until the intercoronary pressure gradient exceeds 70 mm Hg. Clearly, in the normal coronary circulation such intercoronary pressure gradients are not likely to occur even when a coronary artery is chronically instrumented.[39] Consequently, alterations in coronary artery blood flow measured with a flow probe around the proximal artery are virtually identical to the increments in myocardial tissue blood flow measured with radioactive microspheres even under conditions of moderate coronary artery stenosis, which produces an intercoronary pressure gradient of <50 mm Hg.[39] In addition, nearly identical treadmill exercise–induced increases in blood flow have been observed with simultaneous flow probe (to 321% of resting values) and microsphere (to 315% of resting values) measurements in swine.[40] In our laboratory, we previously observed that the vascular resistance responses to dopamine receptor stimulation were similar when measured with a Doppler coronary flow probe or radioactive microspheres.[33] Finally, we observed in 5 swine that coronary artery blood flow increased from 40±3 mL/min at rest to 71±6 mL/min (176±8% of resting values) during exercise at 3 to 4 km/h, while simultaneous microsphere measurements demonstrated an increase in myocardial blood flow from 1.52±0.07 $mL \cdot min^{-1} \cdot g^{-1}$ at rest to 2.82±0.32 $mL \cdot min^{-1} \cdot g^{-1}$ (184±18% of resting values) (authors' unpublished data, 1998). All these studies clearly indicate that under conditions of normal arterial inflow, no discernible collateral blood flow occurs in chronically instrumented hearts, so that the maintained $cvPO_2$ in swine during exercise cannot be explained by the presence of collateral blood flow.

Adrenergic Control of Coronary Blood Flow and Myocardial O_2 Extraction During Treadmill Exercise

α-Adrenoceptors

Although a decrease in $cvPO_2$ during exercise has been proposed to represent a metabolic error signal,[1,10] it is at least in part due to α-adrenergic vasoconstriction,[7,9,11] indicating that an increase in α-adrenergic coronary vasoconstriction can compete with metabolic vasodilation during exercise in dogs. There are some conflicting reports regarding the functional significance of α-adrenergic receptors in the porcine coronary resistance vessels. Thus, Schulz et al[28] infused the selective α_1- and α_2-receptor agonists methoxamine and BHT-933, respectively, into the coronary artery of open-chest vagotomized and β-blocked swine, while coronary blood flow was maintained constant to prevent metabolic counterregulatory mechanisms from masking α-adrenergic constriction. Methoxamine had no effect on coronary artery pressure, whereas BHT-933 produced minimal increases, suggesting that no significant α_1- and minimal α_2-receptors exist in porcine coronary resistance vessels.[28] In contrast, a recent study reported that gallbladder distension resulted in significant reflex-mediated coronary vasoconstriction, resulting in a decrease in coronary blood flow that was amenable to α-adrenergic blockade with phentolamine[41]; the α-receptor subtype responsible for the contraction was not investigated but, on the basis of a report by Schulz et al,[28] would likely be of the α_2 subtype. The observation in the present study that nonselective α-blockade with phentolamine had no influence on coronary vasomotor tone during exercise is consistent with the concept that the predominant α-receptor involved in coronary resistance vessel constriction during exercise is of the α_1 type,[2,9,11] which swine appear to lack.[28] In addition to adrenoreceptors of both the α_1 and α_2 subtypes that are

located on postjunctional vascular smooth muscle cells, α_2-adrenoreceptors are also found prejunctionally, where they inhibit the release of catecholamines from sympathetic nerve endings.[42] Consequently, phentolamine could have increased coronary blood flow and, hence, increase cvP_{O_2} and decrease O_2 extraction indirectly by increasing vascular β-adrenoceptor stimulation. The observation that phentolamine, either in the absence or presence of β-adrenoceptor blockade, had no effect on cvP_{O_2} or O_2 extraction suggests that prejunctional α_2-mediated control of catecholamine release is of minor importance in the porcine coronary circulation.

Although α-adrenergic blockade had no effect on the relation between $M\dot{V}O_2$ and MD_{O_2} or cvP_{O_2}, α-adrenergic blockade produced an upward rotation of the relation between $M\dot{V}O_2$ and coronary blood flow (Figure 2). The latter was necessitated by the α-adrenoceptor blockade–induced blunting of the exercise-induced $12\pm2\%$ increase in Hb. Compared with swine, in dogs, horses, and sheep, O_2 delivery to the myocardium is facilitated by even more prominent increases in Hb concentration (20% to 50%) during exercise and by the resultant increase in the O_2-carrying capacity of arterial blood.[3,4,43–45] The increase in Hb concentration is virtually abolished after splenectomy,[43,44] indicating that exercise elicits splenic contraction that expresses erythrocyte-rich blood into the general circulation. In dogs, splenectomy also necessitated a greater increase in coronary blood flow at comparable levels of $M\dot{V}O_2$ during norepinephrine infusion.[45] In sheep, pretreatment with the nonselective α-adrenergic receptor blocker phenoxybenzamine markedly blunted the increase in Hb concentrations, indicating that contraction of the spleen is mediated by α-adrenergic receptor activation during exercise.[46] Similarly, in the present study, the increase in Hb was prevented by phentolamine, indicating that splenic contraction in swine is also mediated by α-adrenoceptors.

β-Adrenoceptors

Under resting conditions, we observed minimal β-adrenergic vasodilator influences on coronary vasomotor tone, which is in agreement with earlier studies in awake dogs[5,12] and humans.[26] However, a progressive increase in β-adrenergic activity contributed to coronary vasodilation in a feed-forward manner during exercise. Jorgensen et al[27] examined the effect of β-adrenergic blockade with propranolol on coronary blood flow in healthy young adult male human subjects performing upright bicycle exercise. Exercise loads were adjusted to achieve heart rates of 120 bpm during control conditions and after propranolol. At matched heart rates, $M\dot{V}O_2$ levels were similar during control conditions and after β-adrenergic blockade, but coronary blood flow was 25% less after β-adrenergic blockade and was accompanied by an increase in myocardial O_2 extraction. Also, in young male volunteers, β-adrenergic blockade with sotalol (10 mg IV) decreased myocardial blood flow during supine bicycle exercise out of proportion to the reduction of $M\dot{V}O_2$, so that myocardial O_2 extraction rose and coronary sinus O_2 content fell.[26] Finally, in dogs, nonselective β-adrenergic blockade with propranolol also decreased coronary blood flow more than expected from the decrease in $M\dot{V}O_2$, resulting in a significant further increase in myocardial O_2 extraction during treadmill exercise.[5,12] Miyashiro and Feigl[14] demonstrated that the β-adrenoceptor–mediated vasodilation in open-chest dogs produced by infusion of norepinephrine balances the α-adrenergic vasoconstriction. In contrast, in swine that lack significant α-adrenergic vasoconstriction of coronary resistance vessels during exercise, "unopposed" β-adrenergic vasodilation produced vasodilation that matched the increased O_2 demand so that cvO_2 content and P_{O_2} were maintained. Only when β-adrenoceptors were blocked did exercise result in increased O_2 extraction and decreased cvP_{O_2}. The latter possibly served as a metabolic error signal to produce coronary vasodilation during exercise in the presence of β-adrenoceptor blockade.

Parasympathetic Control of Coronary Blood Flow at Rest and During Treadmill Exercise

The coronary resistance vessels are richly innervated by the parasympathetic division of the autonomic nervous system.[1] In dogs pretreated with propranolol and paced to maintain a constant heart rate, stimulation of the vagosympathetic trunk produces coronary vasodilation independent of the cardiac effects of vagal stimulation.[47] The coronary vasodilation produced by vagal stimulation is blocked by atropine and is mimicked by acetylcholine, which involves the release of endothelial nitric oxide in the dog.[47,48] In contrast, the nitric oxide–mediated acetylcholine-induced vasodilation in swine is outweighed by a direct vasoconstrictor effect of acetylcholine, resulting in a net vasoconstrictor response to acetylcholine[29–31] or vagal nerve stimulation.[29] Despite ample evidence that stimulation of the parasympathetic system can influence coronary vasomotor tone, the effects of vagal activity under basal resting conditions are generally considered to be negligible, even during basal resting conditions, when vagal activity is high.[1,30] However, previous studies have been conducted principally in anesthetized animal models, which could have blunted vagal tone.[49] In addition, coronary flow was not related to O_2 consumption, which, in view of potential cardiac effects of vagal inhibition, makes interpretation of these studies difficult. The present study demonstrates that in awake swine, blockade of muscarinic receptors elicited a vasodilator response in the coronary resistance vessels under resting conditions that waned during exercise. Vasodilation was likely the result of increased β-adrenergic activity, since it was fully blocked by the addition of propranolol, so that propranolol alone or in combination with atropine resulted in similar downward shift of the relation between O_2 consumption and coronary P_{O_2} and upward shift of the relation between O_2 consumption and O_2 extraction. Thus, the constrictor influence that was exerted by the parasympathetic nervous system was due to inhibition of β-adrenergic vasodilator activity, so that withdrawal of vagal tone could have contributed to β-adrenergic vasodilation at lower levels of exercise.

It cannot be determined from the present study whether the increased β-adrenergic vasodilation resulted from disinhibition of norepinephrine release at terminal nerve endings within the coronary bed or was due to an increase in circulating catecholamines. There is evidence that vagal stimulation can produce a direct negative inotropic response

in the LV independent of its effects on heart rate and sympathetic activity.[50] In the present study, atropine produced marked increases in LV dP/dt_{max} that could be due to disinhibition of β-adrenoceptor activity or a direct effect on the myocardium. The observation that LV dP/dt_{max} in resting swine after propranolol alone (2240±110 mm Hg/s) was not different from LV dP/dt_{max} observed after combined propranolol and atropine (2370±170 mm Hg/s) suggests that intrinsic muscarinic activity in the resting state decreased contractility primarily via inhibition of β-adrenergic activity, with no discernible direct effect on the myocardium. Therefore, the increase in LV dP/dt_{max} produced by atropine reflects mostly an increase in sympathetic activity. At comparable levels of LV dP/dt_{max}, ie, under resting conditions in the presence of atropine (4370±250 mm Hg/s) versus exercise at 2 and 3 km/h under control conditions (4480±210 and 4900±290 mm Hg/s at 2 and 3 km/h, respectively), atropine still produced an increase in cvP_{O_2}. Furthermore, over the entire range of exercise intensities during control conditions, cvP_{O_2} was not altered despite marked increments in sympathetic activity. Taken together, these findings could be interpreted to suggest that the interaction between the parasympathetic and sympathetic nervous system did not occur at the level of circulating catecholamines but at the level of the resistance vessels. Parasympathetic and sympathetic nerve fiber endings are found at the adventitial-medial border in coronary resistance vessels, indicating that parasympathetic fibers could directly inhibit the release of norepinephrine from the terminal nerve endings in the coronary bed, thereby reducing β-adrenergic vasodilation.[1] Further studies using intracoronary administration of atropine and propranolol are necessary to determine whether the increased β-adrenergic vasodilation was the result of increased circulating levels of catecholamines, a local interaction at the nerve endings in the coronary resistance vessels, or both.

Conclusions

The present study described the MDO_2 and extraction patterns of the LV and its modulation by the autonomic nervous system in awake swine at rest and during treadmill exercise. O_2 extraction and cvP_{O_2} were not altered from resting levels during treadmill exercise at levels up to 85% to 90% of maximum heart rate. The maintained levels of cvP_{O_2} were the result of feed-forward β-adrenergic coronary vasodilation during exercise in conjunction with minimal α-adrenergic vasoconstriction. The β-adrenergic vasodilation was likely due to a direct increase in sympathetic activity but may have been supported at lower levels of exercise by withdrawal of muscarinic receptor–mediated inhibition of β-adrenergic vasodilation.

Although there are a few studies of β-adrenergic control of MDO_2 in humans,[26,27] there are, to our knowledge, no human data regarding α-adrenergic or muscarinic control of vasomotor tone in coronary resistance vessels and their relative contribution to regulation of coronary resistance vessel tone at rest or during exercise. Consequently, concepts of mechanisms of coronary blood flow regulation in the human heart during exercise have, so far, been based largely on exercise data obtained in the dog.[1,2] The present study demonstrates significant interspecies differences in coronary vasomotor response and its autonomic control during exercise, warranting studies of autonomic vasomotor control of coronary resistance vessels in humans.

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