Dobutamine enhances both contractile function and energy reserves in hypoperfused canine right ventricle

KUN DON YI, H. FRED DOWNEY, XIAOMING BIAN, MIN FU, AND ROBERT T. MALLET
Department of Integrative Physiology and Cardiovascular Research Institute, University of North Texas Health Science Center, Fort Worth, Texas 76107-2699

Received 23 May 2000; accepted in final form 1 August 2000

Yi, Kun Don, H. Fred Downey, Xiaoming Bian, Min Fu, and Robert T. Mallet. Dobutamine enhances both contractile function and energy reserves in hypoperfused canine right ventricle. Am J Physiol Heart Circ Physiol 279: H2975–H2985, 2000.—Although the β₁-adrenergic agent dobutamine is used clinically to provide inotropic support to the failing myocardium, it could jeopardize the myocardium by depleting energy reserves. This investigation delineated the contractile and energetic effects of low versus high dobutamine doses in the hypoperfused right ventricular (RV) myocardium. The right coronary artery (RCA) of anesthetized dogs was cannulated for controlled perfusion with arterial blood, and regional RV contractile function was measured. RCA perfusion pressure was lowered from 100 mmHg baseline to 40 mmHg, and flow fell by 54%. At 15-min hypoperfusion, dobutamine was infused into the RCA at either 0.01 (low-dose dobutamine) or 0.06 μg・kg⁻¹・min⁻¹ (high-dose dobutamine) for 15 min. Regional power (systolic segment shortening × isometric developed force × heart rate) stabilized at 63% of baseline during hypoperfusion. Low-dose dobutamine restored power to baseline but did not increase RV myocardial O₂ consumption (MVO₂) and thus increased myocardial O₂ utilization efficiency (O₂UE:power/MV02). At 5 min, high-dose dobutamine enhancement of power was similar to that of low-dose dobutamine, but by 15 min, power and O₂UE fell to untreated levels. Remarkably, low-dose dobutamine tripled cytosolic phosphorylation potential; in contrast, high-dose dobutamine lowered phosphorylation potential to 45% of the untreated value. Analyses of glucose uptake and glycolytic intermediates revealed sustained enhancement of glycolysis by low-dose dobutamine, but glycolysis became limited at glyceraldehyde 3-phosphate dehydrogenase during high-dose dobutamine treatment. In summary, low-dose dobutamine improved mechanical performance and efficiency of the hypoperfused RV myocardium while increasing myocardial energy reserves, but high-dose dobutamine failed to sustain improved function and depleted energy reserves. Dobutamine is capable of improving both contractile function and cellular energetics in the hypoperfused RV myocardium, but dosage should be carefully selected.

phosphorylation potential; phosphocreatine; glycogen; glycolysis; oxygen utilization efficiency

DOBUTAMINE and other β-adrenergic agents powerfully stimulate myocardial contractile function and thus are potentially effective treatments for cardiac insufficiency. Unfortunately, β-adrenergic stimulation of myocardium incurs a cost: by increasing energy demand without a commensurate increase in energy supply, β-adrenergic agents deplete the myocardium of its critically important energy reserves (44, 56). This energy depletion can produce an array of undesirable sequelae, including arrhythmias (26), oxygen wasting (15, 40), and myocardial necrosis (27). Moreover, β-adrenergic agents stimulate the formation of harmful oxyradicals (10, 36, 44). These problems have limited the clinical application of β-adrenergic agents, and the potential utility of these drugs to provide inotropic support for failing myocardium has not been fully realized.

The effects of dobutamine on contractile function and energy reserves in the left ventricular myocardium have been studied extensively. In general, dobutamine increased function but depleted high-energy phosphate compounds in the hypertrophied (1, 24, 55) or hypoperfused (9, 41, 54) left ventricular myocardium of dogs and pigs. Comparatively few studies have examined the hemodynamic and energetic effects of β-adrenergic stimulation in the right ventricular (RV) myocardium. The left and right ventricles have very different workloads, wall tension and structure, coronary flow patterns, perfusion, and metabolic rates (18, 23, 45), so RV responses to inotropic stimulation should not be assumed solely by extrapolation from left ventricular responses. Recently, Greyson et al. (8) reported that intravenous dobutamine increased global but not regional RV contractile function during prolonged right coronary hypoperfusion in pigs without a concordant reduction in myocardial high-energy phosphate content. In contrast, Schwartz et al. (43) recently reported an increase in high-energy phosphates in the porcine right ventricle during isoproterenol infusion in the absence of coronary flow limitation.

We recently demonstrated that β-adrenergically stimulated, hypoperfused left ventricular myocardium can restore its oxygen supply-to-demand balance and maintain its contractile function by increasing its oxygen utilization efficiency (20). In the RV myocardium,
energy metabolites, including ATP, phosphocreatine (PCr), creatine (Cr), and inorganic phosphate (Pi), remained unaltered in the face of moderately severe right coronary hypoperfusion in the absence of β-adrenergic stimulation (12). If the mechanisms that increase oxygen utilization efficiency during β-adrenergic stimulation of the left ventricular myocardium also operate in the right ventricle, it seems possible that energy reserves of the hypoperfused RV myocardium might be maintained or even increased by dobutamine stimulation. This possibility was examined in anesthetized open-chest dogs by lowering right coronary perfusion pressure (RCP) sufficiently to partially compromise regional contractile function and then infusing dobutamine at two different intracoronary concentrations. Global and regional contractile function, regional myocardial consumption of oxygen (MV_{O_2}), glucose and lactate uptake, and the oxygen utilization efficiency (O_{2}UE) were monitored. The myocardium was sampled for measurement of energy metabolites, glycolytic intermediates, and glycogen. At the lower concentration, dobutamine produced sustained increases in regional contractile performance and O_{2}UE and markedly enhanced the cytosolic phosphorylation potential of the hypoperfused RV myocardium. In contrast, the higher dobutamine concentration produced only transient improvements in function, and phosphorylation potential fell sharply. Thus the inotropic and energetic effects of dobutamine in the RV myocardium appear to be heavily dose dependent.

METHODS

Surgical Preparations

Animal experimentation was approved by the Institutional Animal Care and Use Committee of the University of North Texas Health Science Center at Fort Worth and was conducted in accordance with the Guide to the Care and Use of Laboratory Animals (NIH 85–23, Revised 1996). Thirty-nine mongrel dogs of either gender were initially anesthetized with pentobarbital sodium and fentanyl (10 \text{mg/kg} iv) were administered intravenously. The myocardium was exposed via a right thoracotomy in the fourth intercostal space. The pericardium was incised, and the heart was suspended in a pericardial cradle. A Millar catheter-tip transducer was inserted through the right atrial appendage and advanced across the tricuspid valve to measure right ventricular pressure (RVP). The first derivative of RVP (dP/dt) was computed electronically by a Grass model 7P20C differen-tiator.

The right coronary artery (RCA) was isolated near its origin and, after heparin administration (500 \text{U/kg} iv), cannulated with a stainless steel cannula connected to the extracorporeal perfusion system. RCP was controlled by a pressurized reservoir supplied with blood withdrawn from the left femoral artery. A fluid-filled catheter was advanced to the cannula orifice and connected to a pressure transducer for monitoring RCP. Right coronary blood flow (RCBF) was measured electromagnetically with a Carolina Medical Electronics flowmeter and an in-line flow transducer.

Regional Myocardial Function

Within the perfusion territory of the RCA, a pair of piezoelectric crystals were implanted in the midwall of the right ventricle to measure segment length (12, 20). The crystals were placed ~1 cm apart and positioned parallel to the principal axis of shortening in the perfusion territory of the RCA. End-diastolic length (EDL) and end-systolic length (ESL) were measured at the beginning of the positive deflection of the dP/dt record and 20 ms before the peak negative deflection, respectively. Myocardial segment shortening during systole (SS) was expressed as a fraction of EDL; thus SS = [(EDL – ESL)/EDL]. An isometric force transducer was placed 10 mm toward the base of the heart, parallel to the position of the piezoelectric crystals in the perfusion territory of the RCA, to measure RV isometric force. AoP, heart rate (HR), RCFB, RCP, RVP, dP/dt, RV SS, and RV isometric force were recorded on a Grass model 7D eight-channel polygraph. A vein draining the RCA perfusion territory was cannulated to collect venous samples (12).

MV_{O_2} and Lactate and Glucose Uptakes

Coronary arterial and venous samples were collected anaerobically and chilled on ice until analysis. The Po_{2}, PCO_{2}, and pH of each sample were measured with an Instrument Laboratory model 1730 blood gas analyzer, oxygen content was measured with an Instrument Laboratory model 682 Co-Oximeter, and blood glucose and lactate concentrations were measured with a Yellow Springs Instruments model 2300 l-lactate analyzer. Myocardial oxygen consumption (MV_{O_2}) and lactate and glucose uptakes were determined from the product of arteriovenous difference and RCFB (12, 20). An index of contractile power (PI) generated in the RCA perfusion territory was computed as the product of HR, SS, and isometric force (14). O_{2}UE was defined as the ratio of PI to MV_{O_2}.

Right Coronary Perfusion Territory

Because the RCA perfusion territory was biopsied during the protocols, it was not possible to directly measure its mass. Accordingly, the mass of the RCA perfused myocardium was estimated from the baseline RCFB at 100 mmHg perfusion pressure by assuming flow to equal 0.5 \text{ml.min}^{-1}.\text{g}^{-1} (2, 29, 49, 53). With this approach, the mean mass of the right coronary perfusion territory was calculated to be 21 ± 2 g.

Experimental Protocols

Group 1: untreated hypoperfusion (n = 9). The RCA was perfused at a pressure of 100 mmHg for ~30 min to allow hemodynamic variables and regional function to stabilize after the surgical preparation. During this period, blood
Determination of Pi and Phosphorylation State of PCr

Samples from all four groups were extracted on the same day following the protocol for group 1 except that 15 min after RCP was lowered to 38 mmHg, dobutamine was infused into the RCA for an additional 15 min at a rate of 0.01 μg·kg⁻¹·min⁻¹. A portion of the RCA perfusion territory was biopsied as described in group 1.

Group 2: low-dose dobutamine treatment (n = 13). The protocol for group 2 was similar to that of group 1 except that 15 min after RCP was lowered to 41 ± 2 mmHg, dobutamine was continuously infused into the RCA for an additional 15 min at a rate of 0.01 μg·kg⁻¹·min⁻¹. A portion of the RCA perfusion territory was biopsied as described in group 1.

Group 3: high-dose dobutamine treatment (n = 5). The protocol for group 3 was the same as that of group 2 except that 15 min after RCP was lowered to 38 ± 3 mmHg, dobutamine was infused at a higher rate (0.06 μg·kg⁻¹·body mass⁻¹·min⁻¹). A portion of the RCA perfusion territory was biopsied as described in group 1.

Group 4: baseline control (n = 11). This experiment served as time control for the hypoperfusion protocols. After a postsurgical stabilization period of 30 min, the RCA was perfused at baseline pressure of 100 mmHg for 75 min. Blood samples and hemodynamic data were collected at the same time points described in the group 1 protocol. A portion of the RCA perfusion territory was biopsied as described in group 1.

Myocardial Metabolite Analyses

The myocardium in the center of the RCA perfusion territory was biopsied at the completion of each protocol. Immediately after biopsy, the frozen myocardium was quickly immersed in liquid nitrogen and subsequently stored at −90°C until metabolite extraction. Only frozen myocardium compressed between the clamps was used for metabolite assays. The frozen myocardium was ground to a fine powder in a mortar under liquid nitrogen, and energy metabolites, glycolytic intermediates, and sucrose were extracted in four volumes of ice-cold 0.3 N HClO₄, as described previously (12, 49). ATP, PCr, Cr, Pi, glycolytic intermediates, glycogen, and sucrose were measured by colorimetric assays (12, 49). An aliquot of powdered tissue was desiccated to a constant mass at 100°C for determination of dry mass. The appropriate correction factors for dilution and tissue mass were applied. Samples from all four groups were extracted on the same day to prevent artifactual differences in measured metabolites.

Determination of Pi and Phosphorylation State of PCr

PCr phosphorylation potential (ΔPCr/Δ[Cr]Pi) was determined as an index of the cytosolic ATP phosphorylation potential (12, 49, 51). Intracellular Pi, in mM (in mM) was calculated from the following equation

\[
\text{Intracellular Pi} = \frac{1}{\text{Tissue Pi} - \text{ Plasma Pi} \times \text{E_i} \times \text{R_i}} \times \text{[1 - (E_i + R_i)]} \quad (1)
\]

\[
\frac{\Delta \text{PCr}}{\Delta [\text{Cr}] \text{Pi}}
\]

where tissue Pi is the total myocardial Pi content (in μmol/g wet wt), plasma Pi is the concentration of Pi in coronary venous plasma (in mM), Eᵢ is the myocardial extracellular space (in ml/g wet wt), and Rᵢ is the myocardial dry mass.
to-wet mass ratio. Extracellular space was determined as the sucrose distribution space (12).

Cytosolic Redox Metabolites

Right coronary arterial and venous plasma samples (0.5–1.0 ml) collected during the final minute of each experiment were extracted with one volume of 0.6 N HClO4 and neutralized to pH 6.0–7.0 with KOH. The pyruvate and lactate in plasma and myocardial extracts were measured colorimetrically (19, 31). Extracellular concentrations of these compounds were taken as the mean of the respective arterial and venous concentrations, and intracellular concentrations were computed as described above for Pc. Cytosolic redox state was assessed from intracellular lactate-to-pyruvate concentration ratios according to the lactate dehydrogenase equilibrium (32, 51).

Statistical Analyses

All data are expressed as means ± SE. Hemodynamic, functional, and metabolite data were analyzed by one-way ANOVA to determine the differences among groups and by one-way ANOVA for repeated measures to determine the differences between experimental conditions within each group. When significance was found with ANOVA, Student-Newman-Keul’s multiple comparison tests were performed. Statistical significance was assumed at P < 0.05.

RESULTS

RV Hemodynamics and Blood Gases

Hemodynamic variables of groups 1–3 are presented in Table 1. Data are presented for the baseline conditions with RCP at 100 mmHg and after RCP reduction to 38–41 mmHg, which caused RCBF to fall by 54% versus baseline. Although coronary flow fell in lockstep with RCP, hemodynamic variables at 60 and 50 mmHg did not differ among groups 1–3 or from baseline values within each group (data not presented). At ~40 mmHg RCP, AoP, HR, maximum rate of relaxation (−dP/dtmin), and RVP did not differ among the three groups nor from the respective baseline values. During low- and high-dose dobutamine stimulation, RCBF increased by 15–20% but did not differ between groups 2 and 3. Neither low- nor high-dose dobutamine treatment altered AoP, RVP, and −dP/dtmin. Low-dose dobutamine (group 2) did not alter HR, whereas high-dose dobutamine (group 3) significantly increased HR throughout the treatment period. The maximum rate of RV pressure development (+dP/dtmax) fell 13% from baseline during predobutamine hypoperfusion (Table 1). Low-dose dobutamine treatment did not alter +dP/dtmax; however, in group 3, high-dose dobutamine increased +dP/dtmax ~19% versus pretreatment to significantly higher levels than in groups 1 and 2 during the same period. Hemodynamic variables of the time-matched normoperfusion group 4 did not differ from baseline values in groups 1–3.

Table 2 presents coronary venous PO2 and PCO2 (PvO2 and PvCO2, respectively). During moderate hypoperfusion in groups 1–3, PvO2 fell ~21%, whereas PvCO2 increased 12%. Initially, low-dose dobutamine stimulation in group 2 reduced PvO2 slightly; however, PvO2 was not altered, and with continued treatment, PvO2 and PvCO2 remained stable. At 5 min of high-dose dobutamine treatment in group 3, PvO2 significantly decreased, whereas PvCO2 increased compared with the untreated hypoperfused condition. At 15 min of high-dose dobutamine stimulation, PvO2 continued to fall well below the 5-min treatment value, whereas PvCO2 continued to increase, in contrast to low-dose dobutamine treatment.

Table 1. RV hemodynamics

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>RCP, mmHg</th>
<th>AoP, mmHg</th>
<th>HR, beats/min</th>
<th>RCBF, ml·min⁻¹·g⁻¹</th>
<th>RVP, mmHg</th>
<th>+dP/dtmax, mmHg/s</th>
<th>−dP/dtmin, mmHg/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>100</td>
<td>102 ± 2</td>
<td>125 ± 5</td>
<td>0.51 ± 0.02</td>
<td>27 ± 2</td>
<td>435 ± 25</td>
<td>−347 ± 42</td>
</tr>
<tr>
<td>Group 2</td>
<td>100</td>
<td>104 ± 4</td>
<td>128 ± 9</td>
<td>0.50 ± 0.02</td>
<td>27 ± 3</td>
<td>442 ± 20</td>
<td>−345 ± 27</td>
</tr>
<tr>
<td>Group 3</td>
<td>100</td>
<td>105 ± 5</td>
<td>129 ± 7</td>
<td>0.50 ± 0.02</td>
<td>27 ± 2</td>
<td>444 ± 41</td>
<td>−342 ± 20</td>
</tr>
<tr>
<td>Hypoperfusion, predobutamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>40 ± 2</td>
<td>100 ± 4</td>
<td>125 ± 7</td>
<td>0.23 ± 0.03*</td>
<td>26 ± 2</td>
<td>380 ± 19*</td>
<td>−298 ± 39</td>
</tr>
<tr>
<td>Group 2</td>
<td>41 ± 2</td>
<td>107 ± 3</td>
<td>125 ± 6</td>
<td>0.23 ± 0.02*</td>
<td>26 ± 3</td>
<td>390 ± 15*</td>
<td>−286 ± 19</td>
</tr>
<tr>
<td>Group 3</td>
<td>38 ± 3</td>
<td>108 ± 4</td>
<td>137 ± 7</td>
<td>0.23 ± 0.01*</td>
<td>25 ± 2</td>
<td>386 ± 49*</td>
<td>−293 ± 22</td>
</tr>
<tr>
<td>5 min dobutamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>40 ± 2</td>
<td>100 ± 4</td>
<td>125 ± 8</td>
<td>0.23 ± 0.03*</td>
<td>26 ± 2</td>
<td>394 ± 24</td>
<td>−300 ± 40</td>
</tr>
<tr>
<td>Group 2</td>
<td>41 ± 2</td>
<td>109 ± 3</td>
<td>137 ± 8</td>
<td>0.26 ± 0.02+*</td>
<td>28 ± 3</td>
<td>417 ± 27</td>
<td>−347 ± 17</td>
</tr>
<tr>
<td>Group 3</td>
<td>38 ± 3</td>
<td>107 ± 5</td>
<td>162 ± 3††‡</td>
<td>0.29 ± 0.02+*</td>
<td>27 ± 2</td>
<td>475 ± 53†</td>
<td>−332 ± 32</td>
</tr>
<tr>
<td>15 min dobutamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>40 ± 2</td>
<td>101 ± 4</td>
<td>124 ± 8</td>
<td>0.22 ± 0.03*</td>
<td>26 ± 2</td>
<td>390 ± 15</td>
<td>−299 ± 38</td>
</tr>
<tr>
<td>Group 2</td>
<td>41 ± 2</td>
<td>110 ± 3</td>
<td>135 ± 8</td>
<td>0.26 ± 0.02+‡</td>
<td>28 ± 3</td>
<td>425 ± 27</td>
<td>−349 ± 17</td>
</tr>
<tr>
<td>Group 3</td>
<td>38 ± 3</td>
<td>107 ± 5</td>
<td>166 ± 3††‡</td>
<td>0.29 ± 0.02+‡</td>
<td>27 ± 2</td>
<td>472 ± 65†</td>
<td>−335 ± 37</td>
</tr>
</tbody>
</table>

Values are means ± SE. RCP, right coronary perfusion pressure; AoP, mean aortic pressure; HR, heart rate; RCBF, right coronary blood flow; RVP, right ventricular (RV) peak systolic pressure; +dP/dtmax, maximum rate of pressure development; −dP/dtmin, maximum rate of relaxation. Group 1, untreated hypoperfusion, n = 9; group 2, low-dose dobutamine, n = 13; group 3, high-dose dobutamine, n = 6.*P < 0.05 vs. respective baseline value at 100 mmHg; †P < 0.05 vs. pretreatment, same group; ‡P < 0.05 vs. untreated, same period.
**Table 2. Right coronary venous blood gases and myocardial lactate and glucose uptake**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>RCP, mmHg</th>
<th>$P_{O_2}$, mmHg</th>
<th>$P_{CO_2}$, mmHg</th>
<th>Lactate Uptake, μmol·min$^{-1}$·g$^{-1}$</th>
<th>Glucose Uptake, μmol·min$^{-1}$·g$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>100</td>
<td>36.7 ± 1.7</td>
<td>42.0 ± 2.5</td>
<td>0.17 ± 0.02</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Group 2</td>
<td>100</td>
<td>36.9 ± 2.0</td>
<td>42.1 ± 1.9</td>
<td>0.20 ± 0.07</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Group 3</td>
<td>100</td>
<td>36.5 ± 2.7</td>
<td>42.8 ± 1.1</td>
<td>0.17 ± 0.02</td>
<td>0.12 ± 0.03</td>
</tr>
</tbody>
</table>

**Hypoperfusion, pretreatment**

| Group 1         | 40 ± 2    | 29.7 ± 1.8*     | 48.9 ± 1.8*     | 0.04 ± 0.02*                           | 0.18 ± 0.01*                           |
| Group 2         | 41 ± 2    | 28.5 ± 1.5*     | 47.3 ± 2.1*     | 0.03 ± 0.02*                           | 0.18 ± 0.03*                           |
| Group 3         | 38 ± 3    | 28.8 ± 1.1*     | 48.7 ± 2.1*     | 0.03 ± 0.04*                           | 0.18 ± 0.02*                           |

5 min dobutamine

| Group 1         | 40 ± 2    | 29.3 ± 1.8*     | 49.6 ± 2.0*     | 0.04 ± 0.02*                           | 0.17 ± 0.01*                           |
| Group 2         | 41 ± 2    | 26.2 ± 1.2†     | 49.3 ± 2.0*     | −0.02 ± 0.04†                          | 0.25 ± 0.02†                           |
| Group 3         | 38 ± 3    | 25.2 ± 1.8†     | 53.7 ± 3.7†     | −0.04 ± 0.07†                          | 0.25 ± 0.03†                           |

15 min dobutamine

| Group 1         | 40 ± 2    | 29.2 ± 1.6*     | 50.0 ± 2.1*     | 0.03 ± 0.02*                           | 0.17 ± 0.01*                           |
| Group 2         | 41 ± 2    | 26.3 ± 1.2†     | 48.7 ± 2.0*     | −0.02 ± 0.04†                          | 0.25 ± 0.02†                           |
| Group 3         | 38 ± 3    | 15.7 ± 1.3§     | 63.6 ± 5.0§     | −0.28 ± 0.09§                          | 0.29 ± 0.03§                           |

Values are means ± SE. $P_{O_2}$ and $P_{CO_2}$ right coronary venous $P_{O_2}$ and $P_{CO_2}$, respectively. Group 1, untreated hypoperfusion, $n = 9$; group 2, low-dose dobutamine, $n = 13$; group 3, high-dose dobutamine, $n = 6$. *$P < 0.05$ vs. respective baseline value at 100 mmHg; †$P < 0.05$ vs. pretreatment, same group; ‡$P < 0.05$ vs. untreated, same period; §$P < 0.05$ vs. all values.

**Lactate and Glucose Uptakes**

RV uptakes of lactate and glucose are presented in Table 2. Lactate uptake fell markedly in each group during predobutamine hypoperfusion. Low-dose dobutamine elicited a modest net lactate release. At 5 min, high-dose dobutamine produced a similar, modest lactate release; however, by 15 min, lactate release had increased by sevenfold. Glucose uptake increased 33% versus baseline after RCP was lowered to 40 mmHg in groups 1–3. Both doses of dobutamine increased glucose uptake another 30% to roughly twice the baseline values, and glucose uptake in the two dobutamine groups did not differ.

**Regional Myocardial Function**

Contractile function, myocardial lactate and glucose uptakes, and $\text{MV}_{O_2}$ were unaltered at 60 or 50 versus 100 mmHg baseline (data not shown). With the reduction of RCP to ~40 mmHg, SS in all groups fell by ~37% compared with baseline values (Fig. 1A). At 5 min of low-dose dobutamine treatment, SS recovered to baseline values, although there was no concomitant increase in RCBF (Table 1) and $\text{MV}_{O_2}$ (Fig. 2A). The increase in SS was maintained for 15 min. Likewise, high-dose dobutamine initially restored SS to baseline levels; however, as treatment continued, SS fell to pretreatment values. Changes in RV isometric force and PI paralleled those of SS during moderate hypoperfusion and dobutamine infusion (Fig. 1, B and C). Thus isometric force and PI fell during right coronary hypoperfusion. Low-dose dobutamine produced sustained increases in isometric force and PI, but high-dose dobutamine elicited a biphasic response, wherein the initial enhancements of force and PI were lost by 15-min stimulation.

**$\text{MV}_{O_2}$ and oxygen utilization efficiency**

$\text{MV}_{O_2}$ fell 40% from baseline during pretreatment hypoperfusion (Fig. 2A). Low-dose dobutamine did not significantly increase $\text{MV}_{O_2}$ throughout the treatment period, despite increased regional contractile function. In contrast, high-dose dobutamine produced a sustained increase in $\text{MV}_{O_2}$ to the baseline range, although contractile function was increased only transiently. During pretreatment hypoperfusion, $\text{O}_2$UE fell significantly in all three groups (Fig. 2B). During low-dose dobutamine stimulation, the RV myocardium increased its $\text{O}_2$UE above baseline values and sustained this increase for 15 min. High-dose dobutamine treatment increased $\text{O}_2$UE similarly during the first 5 min, but $\text{O}_2$UE later fell to the untreated level due to the decline in PI.

**Energy Metabolism and Phosphorylation Potential**

RV myocardial contents of ATP, PCr, Cr, and P$_i$ are depleted PCr and increased Cr content and P$_i$ concentration. Thus the two concentrations of dobutamine produced opposite effects on RV myocardial energy metabolites compared with normally perfused group 4 time controls. When low-dose dobutamine (group 2) was administered during moderate hypoperfusion, PCr content unexpectedly increased by 55%, Cr content fell concomitantly, and intracellular [P$_i$] tended to decrease. In contrast, high-dose dobutamine (group 3) depleted PCr and increased Cr content and P$_i$ concentration. Thus the two concentrations of dobutamine produced opposite effects on RV myocardial energy metabolites. To further define the effects of dobutamine on myocardial energy reserves, the energy metabolite ratios (PCr) to (Cr) and (PCr) to (ATP) and the PCr phosphorylation potentials were determined (Fig. 4). Low-dose dobutamine increased the (PCr)-to-
[ATP] ratio well above the corresponding ratios in the other groups. Low-dose dobutamine also increased the [PCr]-to-[Cr] ratio more than twofold versus the time control and nontreated hypoperfused groups and threefold versus the myocardium treated with the higher dobutamine dose. Moreover, low-dose dobutamine increased PCr phosphorylation potential, an index of cytosolic ATP phosphorylation potential (27), 2.5-fold above that of the time control and untreated hypoperfusion groups. In striking contrast, the higher dobutamine infusion sharply lowered the PCr phosphorylation potential to 25–30% of the respective values of groups 1 and 4. Thus the two dobutamine doses produced remarkably different effects on cytosolic energy reserves of the hypoperfused canine RV myocardium.

Glycolytic Intermediates and Glycogen

The status of the glycolytic pathway was analyzed in an attempt to delineate the mechanisms producing the disparate contractile and metabolic responses to low- versus high-dose dobutamine. Figure 5 presents glycolytic intermediates as crossover plots, in which metabolite contents in the low- and high-dose dobutamine groups are normalized to the respective contents in the untreated hypoperfused group and plotted in the glycolytic sequence. Both low- and high-dose dobutamine significantly increased all three hexose phosphate intermediates as well as dihydroxyacetone and glyceraldehyde-3-phosphate. However, the effect of the different dobutamine dosages differed beyond the glyceraldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase (GAPDH/PGK) enzyme couple. Low-dose dobutamine treatment did not alter 3-phosphoglycerate, 2-phosphoglycerate, nor phosphoenolpyruvate contents. High-dose dobutamine stimulation significantly lowered all intermediates beyond glyceraldehyde-3-phosphate, indicating that glycolysis had become constrained at the level of GAPDH/PGK.

To further demonstrate the differing effects on glycolysis of the two dobutamine doses, Figure 6 presents a crossover plot, in which intermediate contents in the high-dose dobutamine treatment group are normalized to the respective contents in the low-dose dobutamine treatment group. All glycolytic intermediates beyond GAPDH/PGK were sharply lowered in the high-dose dobutamine treatment group relative to the low-dose dobutamine treatment group.

Myocardial glycogen content (in µmol/g dry weight) did not significantly fall in hypoperfused group 1 (264 ± 23) versus the normally perfused group 4 myocardium (315 ± 31). Glycogen mobilization by dobutamine was dose dependent: low-dose dobutamine (213 ± 13, *P*, 0.05 vs. groups 3 and 4) tended only to deplete the myocardial glycogen reserves relative to group 1, but high-dose dobutamine (171 ± 11, *P* < 0.05 vs. groups 2 and 4) further depleted the myocardial glycogen reserves by 35% versus untreated group 1 (Fig. 5).

Fig. 2. RV myocardial oxygen consumption (MV\(\dot{O}_2\); A) and oxygen utilization efficiency (B). Oxygen utilization efficiency equaled RV regional power index (Fig. 1C) divided by MV\(\dot{O}_2\). Open bars, untreated hypoperfusion (group 1); hatched bars, low-dose dobutamine (group 2); solid bars, high-dose dobutamine (group 3). Treatment groups, time points, and significance symbols are the same as those of Fig. 1.

Fig. 3. RV myocardial energy metabolites. ATP, phosphocreatine (PCr), creatine (Cr), and inorganic phosphate (P\(_i\)) were measured in myocardium freeze clamped at 30 min untreated hypoperfusion (group 1; open bars), 15 min low-dose dobutamine (group 2; hatched bars), 15 min high-dose dobutamine (group 3; solid bars), or at 75-min nonischemic time control perfusion (group 4; cross-hatched bars). Braces, metabolite content (in µmol/g dry wt); brackets, intracellular concentration (in mM). *P* < 0.05 vs. group 1; †P < 0.05 vs. group 2; ‡P < 0.05 vs. group 4.
Cytosolic Redox State

Intracellular lactate and pyruvate concentrations were determined to assess the effects of low- and high-dose dobutamine on cytosolic redox state, i.e., NADH/NAD⁺ (32). Intracellular pyruvate was similar in the three hypoperfused groups (Fig. 7A). In contrast, intracellular lactate increased sharply and dose dependently with dobutamine (Fig. 7B). Intracellular lactate accumulation produced 6.6- and 42-fold increases in the lactate-to-pyruvate ratio during low- and high-dose dobutamine treatment, respectively.

DISCUSSION

This study investigated the effects of dobutamine on regional contractile function, oxygen demand, and cytosolic energy reserves of the canine RV myocardium during coronary hypoperfusion. Functional and metabolic responses of this hypoperfused myocardium to dobutamine heavily depended on the applied concentration of the β-adrenergic agent. Inotropic stimulation with low-dose dobutamine significantly increased RV regional contractile function without a concomitant increase in MV˙O₂. Remarkably, low-dose dobutamine stimulation did not deplete but instead sharply increased high-energy phosphate reserves and the cytosolic phosphorylation potential. On the other hand, a sixfold higher dose of dobutamine produced a biphasic contractile response: RV regional systolic function initially increased but later fell to pretreatment values despite continued dobutamine infusion. High-dose dobutamine also depleted high-energy phosphates, indicating a renewed metabolic supply-demand imbalance.

Fig. 4. Energy metabolite ratios and PCr phosphorylation potential in RV myocardium. Values are from the same experiments as in Fig. 3. Braces, metabolite content (in µmol/g dry wt); brackets, intracellular concentration (in mM). Open bars, untreated hypoperfusion (group 1); hatched bars, 15 min low-dose dobutamine (group 2); solid bars, 15 min high-dose dobutamine (group 3); cross-hatched bars, 75-min nonischemic time control perfusion. *P < 0.05 vs. group 1; †P < 0.05 vs. group 2; ‡P < 0.05 vs. group 4.

Fig. 5. Crossover plots of glycolytic intermediates in RV myocardium. Metabolite contents in untreated hypoperfusion (group 1; ●) are assigned a value of unity; metabolite contents in low-dose dobutamine-treated myocardium (group 2; ○) and high-dose dobutamine-treated myocardium (group 3; ●) are normalized to the respective group 1 contents. GLY, glycogen; G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; FDP, fructose 1,6-bis-phosphate; DAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate. Group 1 contents (in µmol/g dry wt): GLY 264 ± 23 (glucose equivalents), G6P 0.66 ± 0.18, F6P 0.19 ± 0.04, FDP 0.20 ± 0.04, DAP 0.15 ± 0.02, GAP 0.05 ± 0.01, 3PG 0.37 ± 0.11, 2PG 0.17 ± 0.03, PEP 0.07 ± 0.02. *P < 0.05 vs. control group 1; †P < 0.05 vs. group 2.

Fig. 6. Glycolytic crossover plot in dobutamine-treated myocardium. Metabolite contents in low-dose dobutamine-treated myocardium (group 2; ○) are assigned a value of unity, and metabolite contents in high-dose dobutamine-treated myocardium (group 3; ●) are normalized to respective group 2 values. Abbreviations as in Fig. 5. *P < 0.05 vs. group 2.
Both dobutamine doses stimulated glucose metabolism, but glycolysis became limited at the level of glyceraldehyde 3-phosphate dehydrogenase during high-dose dobutamine stimulation.

Effects of Ischemia and Dobutamine on Regional Function and O$_2$ Demand

When RCP was lowered to ~40 mmHg, regional contractile function fell by ~37%, with a concomitant decrease in MVO$_2$. This acute response is typical of the hibernating myocardium, in which contractile function is persistently but reversibly lowered (5, 38), enabling the chronically underperfused myocardium to remain viable despite restriction of its oxygen supply (20). Downregulation of myocardial oxygen demand during decreased oxygen supply without apparent metabolic and pathophysiological consequences of ischemia was observed in this study during predobutamine hypoperfusion. Our findings of decreased RV function during moderate hypoperfusion are consistent with our previous study (12), in which contractile function was maintained during reductions in RCP until a critical level was reached between 30 and 40 mmHg. In the left ventricle (7, 13, 20), the critical perfusion pressure is much nearer the normal resting level, resulting in a linear relationship between left ventricular function and coronary flow as perfusion pressure is lowered. Because the increased RV contractile function produced by low-dose dobutamine was not accompanied by a concomitant increase in MVO$_2$, downregulation of myocardial oxygen demand persisted despite inotropic stimulation. At the higher dobutamine dose, increased RV contractile function was accompanied by an increase in MVO$_2$; however, the myocardium could not sustain increased contractile function for 15 min, indicating renewed oxygen supply-demand mismatch due to β-adrenergic stimulation.

Effects of Dobutamine on Myocardial O$_2$ Utilization Efficiency and Energy Reserves

In the absence of dobutamine, RV myocardial ATP, PCr, and Cr contents, intracellular P$_i$ concentration, and PCr phosphorylation potential were maintained at the respective baselines during RV hypoperfusion, despite a 54% reduction in RCBF and a 31% decline in O$_2$UE. This energetic stability is most likely due to the concomitant reduction of regional contractile function, which lowers energy demand. O$_2$UE was increased throughout treatment with low-dose dobutamine. The increase in regional contractile function, without a concomitant increase in MVO$_2$, may be explained by an increase in the efficiency of energy transfer from total to external mechanical work, as recently demonstrated by Krams et al. (17) in the stunned porcine myocardium treated with a low dose of dobutamine.

In the high-dose dobutamine group, O$_2$UE increased during the initial 5 min of treatment; however, this increase in O$_2$UE was not maintained. Although myocardial oxygen extraction increased in this group, oxidative metabolism was not sufficient to sustain regional contractile function nor cytosolic phosphorylation potential. This unfavorable situation, which may have been exacerbated by the marked chronotropic effect of high-dose dobutamine, was not observed at the lower dose. Yanagi et al. (52) tested the effects of intravenous dobutamine on the hypoperfused (coronary flow 50% of baseline) left ventricular myocardium in open-chest dogs. When dobutamine did not elicit tachycardia, the cytosolic P$_i$-to-PCr ratio did not change, indicating that myocardial energy reserves were maintained. When tachycardia occurred, the P$_i$-to-PCr ratio increased, indicating depletion of energy reserves. Thus the chronotropic response to dobutamine may be the main...

---

**Fig. 7.** Cytosolic redox metabolites. Pyruvate (A) and lactate (B) were measured at 15-min dobutamine infusion in RV myocardium of group 2 (hatched bars) and group 3 (solid bars) and at the same time in group 1 (open bars). Intracellular metabolite concentrations were computed after subtracting extracellular amounts from the respective myocardial contents and were used to determine intracellular lactate-to-pyruvate concentration ratios (C). Data (means ± SE) are from the same experiments as Fig. 1. *P < 0.05 vs. group 1; †P < 0.05 vs. group 2.
The myocardium gradually shifts from fatty acid to glucose as its principal energy source during moderate ischemia (47, 50). Under these conditions, glycolytic flux and glucose uptake are accelerated through the stimulation of glucose uptake and phosphofructokinase activity (30, 33). Similarly, in this study, glucose uptake increased as RCP was lowered and increased even further during low-dose dobutamine treatment. To examine the effects of dobutamine on glycolysis, myocardial glycolytic intermediates were analyzed by crossover plots (16). Low-dose dobutamine elevated the contents of several glycolytic intermediates, indicating increased entry of hexose into the glycolytic pathway. It thus appears that low-dose dobutamine further enhances glucose metabolism in the hypoperfused RV myocardium.

High-dose dobutamine produced a somewhat different glycolytic pattern. All five measured intermediates “upstream” of GAPDH/PGK accumulated, but those intermediates beyond GAPDH/PGK fell sharply compared with the untreated hypoperfused group. During ischemia (28, 39) or in conditions of near-maximal glycolysis (16), NADH accumulates in the cytosol and limits GAPDH flux, which in turn constrains the overall glycolytic rate. Limitation of GAPDH causes intermediates in the first half of the glycolytic sequence to accumulate and depletes intermediates beyond this reaction, producing a crossover in the glycolytic plot (Figs. 5 and 6). The effects of dobutamine on cytosolic NADH redox state were assessed from the intracellular lactate-to-pyruvate concentration ratio, an index of the cytosolic NADH/NAD+ redox state according to the lactate dehydrogenase equilibrium (32, 51). Although both dobutamine doses increased this ratio, high-dose dobutamine produced a much larger increase than low-dose dobutamine. Lactate release increased sharply as high-dose dobutamine treatment was extended beyond 5 min. The near equality of intracellular pyruvate concentrations in the three hypoperfused groups indicated that increased lactate release during high-dose dobutamine resulted from increased cytosolic NADH/NAD+ and not from increased pyruvate concentration. Thus it appears that NADH/NAD+ increased in the cytosol of cardiomyocytes to a much greater extent during high-versus low-dose dobutamine stimulation and that this profound redox shift served to constrain glycolytic flux. This glycolytic limitation may have contributed to the depletion of energy reserves during high-dose dobutamine treatment. On the other hand, enhancement of energy reserves of the low-dose dobutamine-treated myocardium may be due in part to a sustained increase in glycolysis. Hence, it appears that carefully selected, low doses of dobutamine can produce favorable increases in both myocardial performance and energy reserves, but higher concentrations of dobutamine are energetically costly and cannot sustain improved performance.

**Do butamine Enhanced Glycolysis: A Mechanism for Increased Phosphorylation Potential?**

The decreases in PCr and phosphorylation potential elicited by high-dose dobutamine are in accord with studies in moderately ischemic left ventricular myocardium by Zhang et al. (54), who reported reductions in the PCr-to-ATP ratio in open-chest dogs, and Schulz et al. (42), who reported depletion of high-energy phosphates in open-chest pigs. The cytosolic phosphorylation potential has been found to be directly related to myocardial function (3, 21, 22), and it seems likely that the increased phosphorylation potential may have contributed to the sustained improvement in regional contractile performance by low-dose dobutamine. Conversely, during high-dose dobutamine treatment, the myocardium could not adequately meet the increased energy demand imposed by inotropic stimulation, and, consequently, phosphorylation potential fell.

The specific mechanism(s) of dobutamine-induced changes in O2UE were not delineated in this investigation, but the results suggest at least three factors may have contributed to the differences between low- and high-dose dobutamine. First, the two dobutamine doses produced opposite effects on intracellular Pi. In cardiac muscle, Pi lowers mechanical efficiency by dampening myofilament Ca2+ sensitivity, thus lowering active force developed per ATP hydrolyzed (11, 48). Although low-dose dobutamine tended to lower Pi concentration, high-dose dobutamine increased Pi by 70% versus the untreated myocardium. Second, intracellular acidification lowers mechanical efficiency by dampening Ca2+ activated force development by the myofilaments (6, 35). Coronary venous PCO2, an index of intracellular H+ concentration (3), increased during high- versus low-dose dobutamine treatment, indicating intracellular acidification at the higher dose. Third, at highcardia, which occurred during high- but not low-dose dobutamine treatment, indicating intracellular acidification at the higher dose. Both low- and high-dose dobutamine stimulated utilization of glucose, a more oxygen-efficient fuel than fatty acids or lactate (34), but Pi accumulation, intracellular acidosis, and at highcardia may have combined to offset enhancement of O2UE by glucose metabolism during high-dose dobutamine treatment.

**Limitations of Investigation**

In this study, the RCA perfusion territory was biopsied during the experiments; thus the mass of the perfusion area could not be measured directly. We have previously demonstrated (2, 29, 49, 53) that RCBF is ~0.50 ml·min⁻¹·g⁻¹ of tissue at 100 mmHg RCP. Our assumption that baseline RCBF was 0.50 ml·min⁻¹·g⁻¹ for each heart produced an estimated RCA perfusion territory mass of 21 ± 2 g, in good agreement with directly measured values in dogs of similar size (2, 29, 49, 53). Because RCBF varies from dog to dog, this assumption must have produced errors in values normalized per gram of myocardium. These errors, how-
ever, are likely to be random rather than systematic. Indeed, baseline hemodynamic values and regional contractile function at 100 mmHg RCP were not significantly different among the four groups; thus there was no reason to expect systematic differences in the actual baseline flows.

The possibility must also be considered that reductions in RCP altered the mass of the RCA perfusion territory. We recently demonstrated that total occlusion of the left anterior descending coronary artery caused encroachment of normal perfusion into its territory by only ~2 mm (4). In the absence of total occlusion in the present study, such encroachment was probably even more limited. Hence, the reductions in RCP would have produced only modest, inconsequential decreases in the mass of the myocardium perfused by the RCA.

During low-dose dobutamine treatment, the PCr content and cytosolic phosphorylation potential increased appreciably, without the expected fall in the intracellular P\(_i\) concentration. The inequality between the increase in PCr and decrease in P\(_i\) may reflect glycogen mobilization during low-dose dobutamine treatment. P\(_i\) is liberated when the hexose monophosphate components of glycogen are oxidized, which may offset P\(_i\) sequestration in the expanded PCr reserve. Conversely, PCr and glycogen degradation during high-dose dobutamine stimulation produced a less than expected accumulation of P\(_i\). Under these conditions, some of the P\(_i\) may have effluxed into the extracellular space (37) and thereby limited intracellular P\(_i\) accumulation due to the degradation of PCr and glycogen.

The authors gratefully acknowledge the expert technical assistance of Arthur Williams, Jie Sun, Bradley Hart, and Srinath Setty. This study was supported by National Heart, Lung, and Blood Institute Grants HL-35027 and HL-50441. This study was completed by Kun Dong Yi in partial fulfillment of the requirements for the Master of Science Degree at University of North Texas Health Science Center.

REFERENCES


38. Scholz PM, Kedem J, Cheinberg BV, and Weiss HR. The oxygen wasting effect of isoproterenol is altered by chemical denervation and cardiac hypertrophy. Basic Res Cardiol 91: 308–318, 1996.


