Insulin improves contractile function during moderate ischemia in canine left ventricle

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Tune, Johnathan D., Robert T. Mallet, and H. Fred Downey. Insulin improves contractile function during moderate ischemia in canine left ventricle. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1574–H1581, 1998.—This study determined the effects of insulin on myocardial contractile function and glucose metabolism during moderate coronary hypoperfusion. Coronary perfusion pressure (CPP) was lowered from 100 to 60, 50, and 40 mmHg in the left anterior descending coronary artery of anesthetized, open-chest dogs. Regional glucose uptake (GU), lactate uptake, myocardial O2 consumption, and percent segment shortening (%SS) were measured without (n = 12) or with intravenous (4 U/min, n = 12) or intracoronary insulin (4 U/min, n = 6). Glucose metabolites were also measured in freeze-dried biopsies of control heart (n = 6) and hearts treated with intravenous insulin (n = 6) at the completion of the protocol (40 mmHg CPP). GU increased with intravenous and intracoronary insulin (P < 0.01). In all groups, GU was unaffected by reduced CPP, although lactate uptake decreased significantly (P < 0.01). Myocardial O2 consumption fell (P < 0.05) as CPP was lowered in all groups and was not altered significantly by intravenous or intracoronary insulin treatment. Without insulin, %SS decreased 72% (P < 0.05) at 40 mmHg CPP, but in hearts treated with intravenous and intracoronary insulin, %SS was not reduced (P > 0.05). Myocardial glycogen, alanine, lactate, and pyruvate contents were not significantly different in untreated hearts and hearts treated with intravenous insulin. Thus, in moderately ischemic canine myocardium, insulin markedly improved regional contractile function and did not appreciably increase the products of anaerobic glucose metabolism.

glycolysis; myocardial oxygen consumption; myocardial ischemia

INSULIN WAS ADVOCATED as a therapy for acute myocardial infarction >30 years ago, when Sodi-Pallares et al. (24) hypothesized that infusion of a glucose-insulin-potassium (GIK) solution could restore a normal resting transmembrane potential in ischemic myocardium. They found that GIK decreased S-T segment displacement, lessened the incidence of ventricular arrhythmias, and reduced infarction in situ canine myocardium. They also found beneficial electrocardiographic and antiarrhythmic effects in patients with acute myocardial infarction (24). However, in a subsequent clinical study the British Medical Research Council found no significant difference in mortality or incidence of ventricular arrhythmias between GIK-treated and untreated patients (15). Despite these early conflicting results, clinical interest in GIK as a metabolic therapy for myocardial infarction has endured (12), although a clear clinical benefit has not been shown (5).

Laboratory investigations have continued to yield conflicting results on the effects of insulin in severely underperfused myocardium. For example, insulin treatment lessened the decline of creatine phosphate, glycogen, and ATP and lessened the increase of inorganic phosphate in the infarct zone of baboon myocardium 60 min after coronary artery ligation (19). Insulin treatment lessened contractile dysfunction of canine hearts subjected to 4 h of complete coronary artery occlusion (1). In contrast, insulin failed to prevent ischemic contracture and exacerbated the accumulation of tissue lactate in ischemic, isolated working rat hearts (22).

Insulin-stimulated glycolysis is known to increase lactate formation and intensify cellular acidosis during severe ischemia (16). With continued, albeit reduced, coronary flow, the detrimental effects of stimulated glycolysis might be avoided, but the potential benefits of insulin treatment in such a model of moderate coronary hypoperfusion have not been clearly defined. Apstein et al. (2) found that insulin infusion doubled glycolytic flux and completely prevented contracture but did not improve systolic contractile function in moderately ischemic, isolated buffer-perfused rabbit hearts. In contrast, Liedtke et al. (13) reported that insulin decreased mechanical performance and did not increase ATP production when coronary perfusion was reduced 49% in intact working swine hearts. In light of these conflicting reports, we examined the effects of insulin on glucose metabolism and contractile function in moderately ischemic, in situ canine hearts. Regional coronary hypoperfusion was achieved by incrementally decreasing coronary perfusion pressure (CPP) sufficiently to significantly depress contractile function of untreated control hearts. We found that intravenous and intracoronary insulin infusion markedly improved myocardial contractile function without increasing O2 consumption and did not appreciably increase anaerobic glycolytic products, i.e., lactate and alanine, in moderately ischemic canine myocardium.

METHODS

Surgical Preparation

This investigation was approved by the Institutional Animal Care and Use Committee and was conducted in accordance with the Guide for the Care and Use of Laboratory Animals [DHHS Publ. (NIH) 85-23, revised 1985]. Experiments were performed on 37 mongrel dogs (15–30 kg) of either gender. The dogs were anesthetized with pentobarbital sodium (60 mg/ml, 0.5 ml/kg body wt), and supplemental anesthetic was administered during the experiment to maintain stable anesthesia. A tracheotomy was performed, and the lungs were ventilated with room air; positive end-expiratory pressure was held at 2 cmH2O to prevent atelectasis. Arterial blood samples were frequently analyzed for PO2, PCO2, and pH, and ventilation was adjusted to maintain these variables...
within the limits of 80–140 mmHg, 35–45 mmHg, and 7.35–7.45, respectively. Intravenous sodium bicarbonate was administered if required to maintain normal arterial pH when Pco₂ was within these limits. Rectal temperature was maintained at 37–39°C with a circulating H₂O heating pad.

A vinyl catheter was introduced into the thoracic aorta via the right femoral artery to measure mean aortic blood pressure (AoP). A vinyl catheter was also inserted into the right femoral vein for injecting supplemental anesthetic, sodium bicarbonate, and 20% d-glucose, and in groups 2 and 5 (see Experimental Protocols for explanation of groups) another catheter was placed in the left femoral vein for systemic infusion of insulin (Regular Iletin I, Lilly, beef-pork insulin injection, 4 U/min). The left femoral artery was catheterized to supply blood for an extracorporeal system to perfuse the left anterior descending coronary artery (LAD) at controlled pressure.

A left thoracotomy was performed in the fifth intercostal space to expose the heart. A Millar catheter-tipped pressure transducer was inserted into the left atrial appendage and advanced across the mitral valve to measure left ventricular (LV) pressure, and a vinyl catheter was also inserted into the left atrium to measure left atrial pressure. Heart rate and LV developed pressure (LV dP/dt) were obtained from the LV pressure signal with a cardiotachometer and an electronic differentiator, respectively.

The LAD was isolated distal to its first major diagonal branch. After heparinization (500 U/kg) the LAD was cannulated with a stainless steel cannula (3.0 mm OD, 2.2 mm ID) connected to the extracorporeal perfusion system. The anterior interventricular vein was also cannulated, and the coronary venous blood freely drained into a beaker. This venous blood was intermittently reinfused via the right femoral venous catheter. CPP was adjusted with a servo-controlled peristaltic pump and measured through a saline-filled catheter advanced to the orifice of the LAD cannula. In group 3, insulin was infused into the LAD perfusion line through a 22-gauge needle directed against the flow proximal to the stainless steel cannula. CPP, AoP, and left atrial pressure were measured with fluid-filled pressure transducers. Coronary blood flow was measured with an electromagnetic flowmeter and an in-line flow transducer. Coronary blood flow, CPP, AoP, LV pressure, dP/dt, myocardial segment lengths, and heart rate were recorded on a Sensormedics eight-channel polygraph.

Before termination of the experiments, except in groups 4 and 5, Evans blue dye was injected into the coronary perfusion line at a perfusion pressure of 100 mmHg to delineate the coronary perfusion territory. The hearts were fibrillated by electrical stimulation, and the dried tissue was carefully excised and weighed, so that coronary blood flow could be normalized per gram of tissue mass. In biopsy experiments (groups 4 and 5) the weight of the LAD perfusion territory could not be determined. In these experiments the weight of the perfused region was estimated such that coronary flow of each heart would equal the average flow of groups 1–3 at 100 mmHg CPP, which was 1.0 ml·min⁻¹·g⁻¹.

Blood Gas and Lactate Analysis

Coronary arterial blood was anaerobically sampled from the LAD perfusion line, and coronary venous blood from the anterior interventricular vein was anaerobically collected in chilled tubes under mineral oil. Po₂, Pco₂, and pH of these samples were measured by a Corning automated pH/blood-gas analyzer. O₂ content was measured by an Instrumentation Laboratory CO-oximeter, and blood lactate concentrations (mM) were measured by a Yellow Springs Instruments L-lactate analyzer. Rates of O₂ and lactate consumption of the LAD-perfused region were computed as the product of coronary blood flow (normalized per gram tissue mass) and the arteriovenous differences in blood O₂ and lactate contents, respectively.

Arteriovenous Determination of Glucose

Systemic blood glucose concentrations were monitored throughout the experiment by a Glucometer Elite (Bayer Diagnostics Division). These data were obtained within 1 min of sampling and were used to adjust an intravenous infusion of 20% d-glucose to maintain systemic blood glucose concentrations between 70 and 100 mg/dl. Glucose concentrations of coronary arterial and regional coronary venous plasma of groups 1–3 were determined by enzymatic assay (3) in a Beckman DU 640 spectrophotometer at a wavelength of 337 nm. The coefficient of variation for the assay was determined from 12 repetitive measurements of the same plasma sample and equaled 0.45%. Sodium fluoride (10 mg/ml) was added to each blood sample to inhibit glucose uptake by the erythrocytes before centrifugation (10). Blood glucose concentrations of groups 4 and 5 were determined by a Yellow Springs Instruments glucose analyzer. Myocardial glucose uptake was computed from coronary plasma flow per gram tissue mass multiplied by the arteriovenous difference in plasma glucose concentration for groups 1–3 and from coronary blood flow per gram tissue mass multiplied by the arteriovenous difference in blood glucose concentration for groups 4 and 5.

Assessment of Regional Myocardial Function

Piezoelectric crystals were utilized to measure myocardial segment lengths in the LAD perfusion territory of groups 1–5. The crystals were implanted 1 cm apart in the midmyocardial space near the second diagonal branch of the artery and oriented perpendicular to the major axis of the heart. Signals from the piezoelectric crystals were processed by an ultrasonic dimension system and monitored with an oscilloscope. Segment lengths measured at the beginning of the positive deflection of the dP/dt record were considered to reflect end-diastolic length (EDL), and those measured 20 ms before the peak negative deflection of the dP/dt record were considered to reflect end-systolic length (ESL). Myocardial percent segment shortening (%SS) was computed as [EDL – ESL]/EDL × 100.

Experimental Protocols

Group 1: untreated control (n = 6). When surgical preparation was completed, the LAD was perfused at a pressure of 100 mmHg for 45 min to allow stabilization of hemodynamic variables, dP/dt, and regional myocardial function. Blood gases were also measured during this time. After this stabilization period, baseline measurements were obtained at an LAD perfusion pressure of 100 mmHg. CPP was then incrementally lowered to 60, 50, and 40 mmHg and held at each level for 30 min. A 15-min stabilization period was allowed before measurements were obtained at each of the lowered perfusion pressures, and data were collected during the subsequent 15 min.

Group 2: intravenous insulin (n = 6). The protocol for group 2 was similar to that for group 1, except 15 min before baseline measurements at 100 mmHg CPP, an infusion of insulin (4 U/min) was initiated into the left femoral vein. This infusion was continued throughout the protocol.
Group 3: intracoronary insulin (n = 6). The protocol for group 3 was similar to that for group 2, except insulin (4 U/min) was continuously infused into the LAD perfusion line.

Group 4: control biopsy (n = 6). The protocol for group 4 followed that for group 1, except after measurements were obtained at 40 mmHg CPP, a portion of the LAD free wall was biopsied with aluminum Wollenberger tongs to determine myocardial contents of glucose metabolites (glycogen, alanine, lactate, pyruvate). The tongs were precooled in liquid N2, and only frozen myocardium compressed between the clamps, which was ~1 mm thick and weighed ~1 g, was utilized for metabolite assays. Immediately after biopsy the frozen tissue was immersed in liquid N2 and subsequently stored at ~90°C until metabolite extraction.

Group 5: insulin biopsy (n = 6). The protocol for group 5 was similar to that for group 4, except insulin was continuously infused at 4 U/min into the left femoral vein.

Group 6: regional myocardial blood flow (n = 7). To ascertain transmural blood flow and the extent of collateral blood flow at 40 mmHg CPP, seven additional experiments were conducted. The protocol for group 6 was identical to that for group 1, except regional transmural blood flow (n = 7) was determined at the completion of the protocol (40 mmHg CPP) by injection of radioactive microspheres (11.4 ± 0.1 μm diameter) into the LAD perfusion line proximal to a mixing chamber and collection of duplicate reference samples distal to the mixing chamber. Collateral blood flow (n = 4) was also determined by injection of differently labeled microspheres (11.4 ± 0.1 μm diameter) into the left atrium while the LAD was perfused at 40 mmHg from a reservoir containing nonradioactive blood. Microsphere preparation, tissue sectioning, analysis of radiation, and computation of regional myocardial flow followed standard procedures routinely performed in this laboratory (29).

Metabolic Analyses

Frozen myocardium was powdered in a prechilled porcelain mortar under liquid N2. Myocardial metabolites were extracted with 0.6 N HClO4, as previously described by Bergmeyer (3). Glycogen, alanine, lactate, and pyruvate were assayed enzymatically (3) in a Perkin-Elmer Lambda2 dual-wavelength spectrophotometer (measuring wavelength 337 nm, reference wavelength 417 nm, ε = 5.65 cm−1·mM−1). Dry mass was determined in an aliquot of powdered tissue desiccated to constant mass at 100°C. The appropriate correction factors for volumes of dilution and mass of the tissue were applied, and metabolite contents in micromoles per gram wet tissue mass were determined.

Statistical Analyses

Values are means ± SE for control hearts (groups 1 and 4, n = 12) and hearts treated with intravenous insulin (groups 2 and 5, n = 12) and intracoronary insulin (group 3, n = 6). Results were analyzed by one-way repeated-measures (within-animal design) ANOVA. The ANOVAs were performed to determine the effects of decreased perfusion pressure on each treatment and to determine the effects of treatment at each perfusion pressure. Because the animals served in particular subsets of treatment conditions, sources of variance isolated in the analysis reflected differences within each animal, and therefore a within-animal design was appropriate. To meet the assumptions of the parametric one-way ANOVA, animals were assigned randomly, a Kolmogorov-Smirnov test was performed to verify normality, and variability about the group mean was utilized to test for homogeneity. If the assumptions of the ANOVA were not met, a Friedman repeated-measures ANOVA on ranks was performed. When significance (P < 0.05) was found with ANOVA, a Student-Newman-Keuls multiple comparison test was performed. Differences in myocardial metabolite concentrations between groups 4 and 5 were determined by t-test. These analyses were performed and interpreted according to Keppel (11) and Zar (30). Statistical computation was performed by Sigma Stat Statistical Software version 2.0 (Jandel Scientific, San Rafael, CA).

RESULTS

Table 1 presents hemodynamic and contractile function data of groups 1–5 at normal and reduced CPP. Measures of global hemodynamic function, AoP, heart rate, LV peak systolic pressure, maximum rate of LV pressure development, and maximum rate of LV pressure relaxation were not significantly affected by reduced CPP in any group. When CPP was lowered from baseline of 100 mmHg to 60, 50, and 40 mmHg, LAD flow progressively fell with CPP in each group (P < 0.05), indicating that CPP was below the effective autoregulatory range.

Figure 1 shows the effect of lowered CPP on regional percent systolic segment shortening. In untreated control hearts, percent segment shortening was not significantly decreased as CPP was reduced to 60 and 50 mmHg. However, percent segment shortening fell 72% (P < 0.05) in these hearts when CPP was reduced to 40 mmHg. In contrast, percent segment shortening did not significantly decrease at any CPP in intravenous or intracoronary insulin-treated hearts and was significantly greater than that of untreated controls at 40 mmHg CPP.

Myocardial O2 consumption (MVo2) progressively fell as CPP was lowered to 60, 50, and 40 mmHg in untreated hearts and hearts treated with intravenous and intracoronary insulin (Fig. 2). MVo2 was not significantly altered by intravenous or intracoronary insulin treatment at any CPP, although MVo2 tended to be higher with insulin treatment at all CPPs.

Regional myocardial glucose uptake was markedly increased by intravenous and intracoronary insulin treatments at all CPPs (P < 0.05) but was unaffected by reduced CPP in all groups (Fig. 3). In contrast, regional myocardial lactate uptake fell significantly at 60, 50, and 40 mmHg CPP in all three groups (Fig. 4). Lactate uptake was not significantly altered by intravenous or intracoronary insulin treatment at any CPP. Net release of lactate was evident in insulin-treated hearts at 40 mmHg CPP, indicating anaerobic glycolytic metabolism; however, this increase did not attain significance relative to untreated control hearts.

Table 2 presents glucose metabolite data at 40 mmHg CPP in untreated hearts (group 4) and hearts treated with intravenous insulin (group 5). Regional myocardial contents of glycogen, alanine, pyruvate, and lactate did not differ significantly in these two groups, although in insulin-treated hearts, glycogen tended to be higher (P = 0.1). These results indicate that the increased glucose taken up in the insulin-treated hearts did not result in tissue accumulation of products of anaerobic glucose metabolism, i.e., lactate and alanine. Glycogen might have increased, but the small differ-
A cardiac flow ratio was 0.77 in the regions, respectively. The mean subendocardial-to-subepicardial, midmyocardial, and subendocardial relaxation, respectively. Values are means ± SE from untreated hearts (groups 1 and 4, n = 12) and hearts treated with intravenous insulin (groups 2 and 5, n = 12) and intracoronary insulin (group 3, n = 6). CPP, left anterior descending coronary artery perfusion pressure; AoP, mean aortic pressure; HR, heart rate; CBF, left anterior descending coronary artery blood flow; dP/dt\text{max}, maximum rate of pressure development and relaxation, respectively. *P < 0.05 vs. 100 mmHg CPP, same treatment group.

**Table 1. Hemodynamic variables at normal and reduced CPP in untreated, intravenous insulin-treated, and intracoronary insulin-treated hearts**

<table>
<thead>
<tr>
<th></th>
<th>100 mmHg CPP</th>
<th>60 mmHg CPP</th>
<th>50 mmHg CPP</th>
<th>40 mmHg CPP</th>
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<tr>
<td><strong>Untreated</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AoP, mmHg</td>
<td>103 ± 3</td>
<td>105 ± 4</td>
<td>102 ± 4</td>
<td>100 ± 4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>172 ± 6</td>
<td>170 ± 5</td>
<td>173 ± 5</td>
<td>169 ± 7</td>
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<tr>
<td>CBF, ml·min(^{-1})·g(^{-1})</td>
<td>0.99 ± 0.03</td>
<td>0.59 ± 0.05*</td>
<td>0.46 ± 0.06*</td>
<td>0.31 ± 0.05*</td>
</tr>
<tr>
<td>LVP\text{max}, mmHg</td>
<td>111 ± 5</td>
<td>111 ± 5</td>
<td>108 ± 5</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>dP/dt\text{max}, mmHg</td>
<td>2,000 ± 300</td>
<td>2,100 ± 200</td>
<td>1,900 ± 200</td>
<td>1,900 ± 200</td>
</tr>
<tr>
<td>dP/dt\text{min}, mmHg</td>
<td>−2,300 ± 300</td>
<td>−2,300 ± 300</td>
<td>−2,200 ± 200</td>
<td>−2,100 ± 200</td>
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<tr>
<td><strong>Intravenous insulin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AoP, mmHg</td>
<td>113 ± 6</td>
<td>110 ± 6</td>
<td>103 ± 7</td>
<td>98 ± 7</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>157 ± 6</td>
<td>161 ± 8</td>
<td>157 ± 9</td>
<td>161 ± 6</td>
</tr>
<tr>
<td>CBF, ml·min(^{-1})·g(^{-1})</td>
<td>0.87 ± 0.06</td>
<td>0.66 ± 0.04*</td>
<td>0.52 ± 0.04*</td>
<td>0.40 ± 0.03*</td>
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<tr>
<td>LVP\text{max}, mmHg</td>
<td>126 ± 6</td>
<td>125 ± 8</td>
<td>116 ± 8</td>
<td>118 ± 6</td>
</tr>
<tr>
<td>dP/dt\text{max}, mmHg</td>
<td>2,200 ± 300</td>
<td>2,100 ± 300</td>
<td>2,100 ± 400</td>
<td>2,000 ± 300</td>
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<tr>
<td>dP/dt\text{min}, mmHg</td>
<td>−2,500 ± 200</td>
<td>−2,500 ± 200</td>
<td>−2,400 ± 300</td>
<td>−2,200 ± 300</td>
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<tr>
<td><strong>Intracoronary insulin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AoP, mmHg</td>
<td>106 ± 4</td>
<td>106 ± 5</td>
<td>105 ± 5</td>
<td>107 ± 7</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>168 ± 10</td>
<td>169 ± 10</td>
<td>172 ± 12</td>
<td>175 ± 15</td>
</tr>
<tr>
<td>CBF, ml·min(^{-1})·g(^{-1})</td>
<td>1.12 ± 0.19</td>
<td>0.74 ± 0.06*</td>
<td>0.61 ± 0.05*</td>
<td>0.41 ± 0.05*</td>
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<tr>
<td>LVP\text{max}, mmHg</td>
<td>112 ± 6</td>
<td>111 ± 7</td>
<td>111 ± 7</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>dP/dt\text{max}, mmHg</td>
<td>1,900 ± 100</td>
<td>2,200 ± 200</td>
<td>2,100 ± 200</td>
<td>2,000 ± 147</td>
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<tr>
<td>dP/dt\text{min}, mmHg</td>
<td>−2,100 ± 200</td>
<td>−2,100 ± 200</td>
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<td>−2,000 ± 190</td>
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</table>

DISCUSSION

This study was designed to determine the effects of insulin on myocardial glucose metabolism and contractile function during moderate ischemia. There were two important findings in this study. 1) Relative to untreated control hearts, intravenous and intracoronary insulin treatments markedly increased regional glucose uptake and improved segment shortening, i.e., contractile function, during moderately severe ischemia without significantly increasing MV\(\text{O}_2\). 2) Intravenous insulin administration did not significantly in-
crease myocardial contents of glycogen, alanine, lactate, or pyruvate relative to untreated control hearts in moderately underperfused myocardium. Thus the potentially detrimental accumulation of anaerobic glycolytic products, particularly lactate, was avoided during insulin administration, and contractile function improved in this model of moderate ischemia.

Insulin Treatment and Myocardial Ischemia

GIK as a therapeutic intervention for myocardial infarction has been studied for many years (21, 24, 26). Clinical results have been variable (15, 18, 21, 25), so this therapy is not generally recommended for patients with acute myocardial infarction (5, 18). GIK has also been evaluated for other clinical conditions. Bradley and Branthwaite (5) found that GIK did not improve total LV function after open-heart surgery. However, McDaniel et al. (14) observed an increase in myocardial contractile function in patients treated with GIK during cardiac catheterization for coronary artery disease. In these patients, ischemia was variable and not as severe as in patients with myocardial infarction.

In experimental studies, results have also been inconsistent. Opie et al. (19, 20) found that GIK ameliorated the decline in high-energy reserves after coronary artery occlusion in canine and baboon hearts. In contrast, Rovetto et al. (22) found that insulin did not improve high-energy phosphate levels during low-flow ischemia in isolated, working rat hearts. More recently, Cave et al. (6) found that glucose and insulin treatment increased glycolytic ATP production of isolated, blood-perfused rat hearts subjected to low-flow ischemia. With regard to contractile function, Ahmed et al. (1) reported that insulin treatment lessened ventricular dysfunction relative to untreated hearts during 4 h of complete coronary artery occlusion in anesthetized dogs. In contrast, insulin failed to prevent ischemic contracture and caused a further increase in tissue lactate in isolated, working rat hearts studied by Rovetto et al. As expected, Eberli et al. (7) observed a marked fall in LV developed pressure (from 95 to 5 mmHg) when CPP was reduced to 8 mmHg in isolated rabbit hearts. Glucose and insulin infusion lessened this decline to 23 mmHg, and the improved function was associated with increased coronary flow and O2 consumption. When this increase in coronary flow was prevented at greatly reduced CPP, glucose and insulin treatment still resulted in ~17% improvement in LV developed pressure although O2 consumption did not change.

Discrepancies regarding the beneficial effects of insulin treatment may be attributed, at least in part, to varying degrees of ischemia, which would affect accumulation of lactate (16, 22) and delivery of insulin and glucose to ischemic myocardium (25). To avoid these problems, two previous studies examined the effect of insulin and glucose treatment in moderately underperfused myocardium. Apstein et al. (2) found that glucose and insulin infusion did not increase contractile function during moderate ischemia in isolated, buffer-perfused rabbit hearts, although functional recovery

Table 2. Myocardial contents of glucose metabolites at 40 mmHg CPP in untreated and intravenous insulin-treated hearts

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Intravenous insulin</th>
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<tbody>
<tr>
<td>Glycogen, µmol glucose/g wet tissue</td>
<td>42.2 ± 7</td>
<td>58.9 ± 9</td>
</tr>
<tr>
<td>Alanine, µmol/g wet tissue</td>
<td>0.70 ± 0.2</td>
<td>0.61 ± 0.2</td>
</tr>
<tr>
<td>Lactate, µmol/g wet tissue</td>
<td>1.6 ± 0.5</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Pyruvate, µmol/g wet tissue</td>
<td>0.09 ± 0.03</td>
<td>0.05 ± 0.02</td>
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</table>

Values are means ± SE from untreated (group 4, n = 6) and intravenous insulin-treated (group 5, n = 6) hearts. No statistically significant differences were noted between the 2 groups for any variable.
during reperfusion was improved. Liedtke et al. (13) found that insulin-stimulated glucose metabolism was unable to compensate for the reduction in oxidative metabolism and produced a more pronounced reduction in mechanical performance when coronary flow was reduced 49% in extracorporeally perfused swine hearts. The results of these studies are surprising in light of reports of improved contractile performance in more severely ischemic myocardium. They are also at odds with our findings that insulin treatment prevented a significant fall in contractile function during moderate regional ischemia. One reason for these discrepancies may be that the coronary perfusate of these previous studies was either Krebs-Henseleit buffer (2) or Krebs-Henseleit buffer containing a small percentage of erythrocytes (13). Although significant coronary flow was maintained during their “moderately ischemic” conditions, myocardial O2 delivery was more severely compromised than in our blood-perfused, in situ canine hearts.

We found that myocardial contractile function was markedly improved with insulin treatment, whereas \( \text{MV}_2 \) was not significantly increased, as observed by Eberli et al. (7) under more severely ischemic conditions. Thus myocardial O2 demand was not elevated by insulin, although function increased. This reflects an apparent increase in O2 utilization efficiency (23). In contrast, catecholamine infusion during ischemia would also increase myocardial contractile function but at the expense of increased O2 demand and reduced O2 utilization efficiency (23). Coronary blood flow was not significantly increased with insulin infusion, so enhanced contractile function at reduced CPP was not due to increased O2 supply. Also, tissue lactate was not increased by insulin infusion at 40 mmHg CPP. Thus the detrimental effects of lactate accumulation on contractile function were avoided in this model of moderate ischemia.

Insulin Treatment and Myocardial Energy Metabolism

To evaluate myocardial glucose metabolism, mass balance was calculated in each individual experiment. In intravenous insulin-treated hearts, mean glucose uptake and \( \text{MV}_2 \) were 0.46 ± 0.09 \( \mu \text{mol}\cdot\min^{-1}\cdot\text{g}^{-1} \) and 5.12 ± 0.7 \( \text{ml}\cdot\min^{-1}\cdot\text{100 g}^{-1} \), respectively, at 40 mmHg CPP. It was considered likely that all this \( \text{MV}_2 \) was utilized to oxidize glucose, since glucose uptake was markedly elevated and the myocardium is known to preferentially metabolize glucose during ischemia (17). Furthermore, insulin is known to actively stimulate myocardial glucose metabolism and markedly inhibit fatty acid metabolism (20). If it is assumed that all the \( \text{MV}_2 \) was utilized to oxidize glucose, then an average of 0.31 ± 0.4 \( \mu \text{mol}\) glucose-\( \text{min}^{-1}\cdot\text{g}^{-1} \) was oxidized by intravenous insulin-treated hearts. This accounts for 70% of glucose uptake per minute at this CPP. Lactate production accounts for another 3% of the glucose uptake, i.e., 0.02 ± 0.01 \( \mu \text{mol}\) glucose-\( \text{min}^{-1}\cdot\text{g}^{-1} \). The remaining glucose uptake (0.13 ± 0.05 \( \mu \text{mol}\) glucose-\( \text{min}^{-1}\cdot\text{g}^{-1} \)) was most likely directed to glycogen synthesis, since alanine and pyruvate levels were not affected by intravenous insulin treatment (Table 2). If glycogen synthesis remained constant throughout the 105-min period of insulin infusion, then 13.5 \( \mu \text{mol} \) of glucose would have been incorporated into the myocardial glycogen store, an amount nearly equal to the difference in the mean glycogen contents between intravenous insulin-treated and untreated hearts. However, myocardial glycogen content was measured only at 40 mmHg CPP, and it must be acknowledged that changes in glycogen synthesis throughout the protocol could not be determined.

In the intracoronary insulin-treated hearts, myocardial glucose uptake averaged 0.62 ± 0.13 \( \mu \text{mol}\cdot\min^{-1}\cdot\text{g}^{-1} \) at 40 mmHg CPP. If all the O2 consumed per minute (5.29 ± 0.8 \( \text{ml}\cdot\min^{-1}\cdot\text{100 g}^{-1} \)) is assumed to oxidize glucose, then 0.35 ± 0.05 \( \mu \text{mol}\) glucose-\( \text{min}^{-1}\cdot\text{g}^{-1} \) was oxidized at this CPP. The lactate released by these hearts (0.20 ± 0.16 \( \mu \text{mol}\) glucose-\( \text{min}^{-1}\cdot\text{g}^{-1} \)) accounts for another 0.10 ± 0.08 \( \mu \text{mol}\) glucose-\( \text{min}^{-1}\cdot\text{g}^{-1} \) of glucose uptake. The remaining glucose uptake (0.17 ± 0.04 \( \mu \text{mol}\) glucose-\( \text{min}^{-1}\cdot\text{g}^{-1} \)) was most likely directed to glycogen synthesis. If glycogen synthesis remained constant throughout the period of intracoronary insulin infusion, myocardial glycogen content would have increased by 18 \( \mu \text{mol} \) glucose equivalents/g. However, myocardial glycogen content was not measured in the intracoronary insulin-treated hearts.

In untreated control hearts, glucose and lactate uptake were 0.22 ± 0.04 and 0.04 ± 0.08 \( \mu \text{mol}\cdot\min^{-1}\cdot\text{g}^{-1} \), respectively, at 40 mmHg CPP. \( \text{MV}_2 \) in these hearts was 4.2 ± 0.7 \( \text{ml}\cdot\min^{-1}\cdot\text{100 g}^{-1} \). If all the glucose and lactate taken up per minute is assumed to have been fully oxidized, then 3.65 ± 0.7 \( \mu \text{mol}\) O2-\( \text{min}^{-1}\cdot\text{100 g}^{-1} \) (87% of the measured \( \text{MV}_2 \)) was utilized to metabolize these substrates. The remaining 13% of the O2 consumed (0.55 \( \text{ml}\)\( \text{min}^{-1}\cdot\text{100 g}^{-1} \)) at this CPP was most likely used to metabolize other fuels, e.g., fatty acids and endogenous triglycerides. The average glycogen content in these hearts was 42.2 ± 7 \( \mu \text{mol}\) glucose/g wet tissue, in good agreement with the reported glycogen content of 39.3 ± 2 \( \mu \text{mol}\) glucose/g wet tissue in nonischemic canine myocardium (20). These data indicate that reducing CPP to 40 mmHg did not significantly alter myocardial glycogen utilization of the untreated hearts.

From this analysis, insulin treatment significantly increased glycolytic flux and increased the amount of glucose oxidized by 40%. The increased proportion of energy supplied by the metabolism of glucose would have led to a more efficient utilization of O2, since oxidation of glucose requires less oxygen per ATP produced than does oxidation of fatty acids (17). Glucose uptake was unaltered as CPP was lowered from 100 to 60, 50, and 40 mmHg in each of the three groups, despite appreciable differences in glucose uptake due to insulin at each perfusion pressure. These results in the in situ canine heart preparation confirm recent reports of studies in isolated perfused rat (4) and guinea pig (8) heart preparations in which glucose uptake was essentially unchanged as CPP was lowered sufficiently to produce moderately severe ischemia.
Insulin Enhancement of Myocardial Contractile Function

Insulin-stimulated glycolytic flux could be expected to enhance myocardial contractile function in moderately underperfused myocardium. Cave et al. (6) concluded that glycolytic ATP production, and not oxidative phosphorylation, protected isolated rat hearts against diastolic dysfunction. Xu et al. (28) proposed that during periods of ischemia and/or hypoxia ATP generated by sarcoplasmic reticulum (SR)-associated glycolytic enzymes stimulates SR Ca$^{2+}$-ATPase and increases Ca$^{2+}$ transport. Thus insulin-stimulated glycolytic flux would also enhance SR Ca$^{2+}$-ATPase activity, increase uptake and release of Ca$^{2+}$ by the SR, and improve contractile function. Evidence also suggests that insulin can be internalized by target cells and can activate the myocardial SR Ca$^{2+}$-ATPase by binding directly to SR membranes (9). Delineation of the specific mechanism of insulin enhancement of cardiac inotropism merits further research.

Intracoronary vs. Intravenous Insulin Infusion

Intracoronary and intravenous infusion of insulin were studied in this investigation. Intracoronary insulin infusion produced a greater elevation of intracoronary insulin concentration but did not profoundly increase myocardial glucose uptake or produce a significant increase in lactate release compared with intravenous insulin. However, the lactate released by the hearts treated with intracoronary insulin tended to be higher, which could explain why intravenous insulin was more effective than intracoronary insulin at improving regional segment shortening. Thus it would appear that 4 U/min intravenous insulin infusion produced a maximal increase in myocardial glucose uptake, which was not further enhanced by an increased intracoronary insulin concentration in intracoronary insulin-treated hearts. Intravenous insulin did improve regional segment shortening relative to intracoronary insulin. This contrasting effect of insulin is most likely due to increased anaerobic metabolism of glucose in hearts treated with intracoronary insulin (Fig. 4). Although myocardial glycogen content was not measured in hearts treated with intracoronary insulin, the incremental increase in net lactate release accounts for the excess glucose uptake by these hearts. There were no other hemodynamic, cardiac function, or metabolic differences between the effects of intravenous and intracoronary insulin.

Use of Labeled Precursors to Evaluate Glucose Metabolism

In this study, regional myocardial glucose metabolism was assessed from measurements of glucose and lactate uptake, MV$_{O_2}$, and myocardial products of glucose metabolism. The use of radiolabeled precursors might have provided a more direct means of measuring myocardial glucose metabolism and has been successfully applied in isolated heart preparations (4, 8). However, radiolabeled precursors could not be used to study glucose metabolism in this investigation because of several factors that complicate the use of labeled precursors in whole-animal preparations: 1) To obtain a sufficiently high specific activity of the precursor, very large amounts of radioactivity would have to be infused into the dog, since the dog has large plasma and extracellular fluid volumes containing high concentrations of endogenous glucose. 2) Most of the labeled glucose and the labeled products of glucose metabolism would enter the systemic circulation and be metabolized by tissues elsewhere in the body, generating other labeled substances that could be metabolized by the myocardium. 3) Erythrocytes are heavily dependent on glycolysis for energy production and could consume significant amounts of labeled glucose and produce large amounts of labeled lactate in the coronary circulation, even before the labeled glucose reaches the myocardies. 4) As labeled glucose is consumed by the heart and other organs, its specific activity in the blood would fall, necessitating a continuous, controlled infusion of labeled glucose to maintain precisely the specific activity. These factors present a formidable challenge to the use of radiolabeled glucose in the regionally perfused, in situ canine myocardium.

Regional Myocardial Blood Flow

A transmural gradient of coronary blood flow across the LAD-perfused region was detected at 40 mmHg CPP. Myocardial glucose and lactate uptake and MV$_{O_2}$ were computed from respective arteriovenous content differences times LAD flow measured by an electromagnetic flowmeter in the extracorporeal circuit and thus reflect average transmural values. The modest transmural flow gradient at 40 mmHg CPP indicates that large transmural differences in metabolism were not observed in this process. Collateral blood flow to the LAD region was negligible, and thus measurements of glucose and lactate uptake and MV$_{O_2}$ computed from arteriovenous differences and LAD flow were not affected by collateral flow at reduced CPP. Although collateral flow was not measured in insulin-treated hearts, CPP was controlled similarly, so the pressure gradients for collateral flow were similar in untreated and insulin-treated hearts. Thus it is very unlikely that an increased supply of O$_2$ and substrates via collateral vessels could have accounted for increased contractile function in insulin-treated hearts.

To accurately measure regional myocardial uptake or release of substances from the product of regional blood flow times the regional arteriovenous difference, the sampled venous blood must drain exclusively from the region of interest. Vinten-Johansen et al. (27) evaluated the extent of contributions from non-LAD-perfused tissue to the interventricular venous drainage. They injected $^{51}$Cr-labeled red blood cells systemically, while the LAD was perfused with nonradioactive blood. Minimal radioactivity in blood taken from the anterior interventricular vein demonstrated that this venous blood originated almost exclusively from LAD-perfused myocardium. This was true even when they reduced LAD perfusion pressure to 40 mmHg. Thus it is very
unlikely that regional uptake data reported here were affected significantly by sampling venous blood from non-LAD-perfused tissue.

In conclusion, in moderately ischemic canine myocardium, insulin markedly improved regional myocardial contractile function and did not appreciably increase the products of anaerobic glucose metabolism. Insulin stimulated glucose uptake, glycolytic flux, and increased glucose oxidization. When the potential detrimental effects of insulin-stimulated glucose metabolism are avoided during moderate ischemia, insulin treatment increases contractile function without significantly elevating myocardial O2 demand.

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