The Relative Importance of Myocardial Energy Metabolism Compared With Ischemic Contracture in the Determination of Ischemic Injury in Isolated Perfused Rabbit Hearts

Jean-Louis J. Vanoverschelde, Marc F. Janier, Steven R. Bergmann

Abstract The mechanical effects of ischemic contracture may be important in the development of irreversible cellular damage as it increases mechanical stress on sarcolemmal membranes and restricts endocardial perfusion. To assess the relative importance of these mechanical effects compared with decreased energy supply in the development of irreversible injury, the effects of inhibiting ischemic contracture with 2,3-butanedione monoxime (BDM), an agent that disrupts excitation-contraction coupling, were delineated in isovolumically contracting isolated rabbit hearts. Administration of 20 mmol/L BDM in 12 hearts subjected to 60 minutes of low-flow ischemia prevented ischemic contracture (left ventricular end-diastolic pressure [LVEDP], 12±3 compared with 48±14 mm Hg in 20 control hearts; P<.001), reduced membrane damage (creatine kinase [CK] release, −54% compared with control hearts; P<.05), and enhanced functional recovery during reperfusion (left ventricular developed pressure [LVDP], 86±10% of baseline compared with 56±23% in control hearts; P<.01). These observations were not related to increased intracavitary pressure and its effects on flow distribution, since venting the left ventricle in additional hearts did not result in improved function during reperfusion. Although it would be tempting to conclude that BDM protected ischemic myocardium by preventing ischemic contracture, administration of BDM was also associated with reduced depletion of ATP during ischemia, perhaps related to diminished energy demand. To distinguish between the relative importance of inhibiting contracture from provision of adequate energy, the period of ischemia was extended to 120 minutes. BDM still prevented ischemic contracture (LVEDP, 10±6 mm Hg) and preserved ATP stores, but it did not prevent membrane damage (CK release, 483±254 U/g dry weight) or contractile failure during reperfusion (LVDP, 68±7% of baseline). In contrast, increasing the rate of anaerobic glycolysis during ischemia by doubling glucose and insulin in the presence of BDM markedly decreased membrane damage (CK release, 114±72 U/g dry weight; P<.05) and contractile failure during reperfusion (LVDP, 88±7% recovery of baseline; P<.01). These results suggest that insufficient energy production is primarily responsible for myocardial ischemic damage, whereas mechanical effects of ischemic contracture appear to play only a minor role. (Circ Res. 1994;74:817-828.)

Key Words • anaerobic glycolysis • no-reflow phenomenon • 2,3-butanedione monoxime

In the evolution of cell death induced by ischemia, a number of biochemical and structural alterations occur sequentially. Despite intense study, the molecular factors responsible for irreversible injury have not yet been fully elucidated. One element contributing to the irreversibility of injury is the development of focal deficits or breaks in the sarcolemma of the ischemic myocytes.1 Once these have developed, myocytes become osmotically fragile, lose low molecular weight molecules, and accumulate extracellular electrolytes such as Na+ and Ca2+.3,4

Recent reports have suggested that ischemic contracture could be an important factor in the development of irreversible injury.3-5 In isolated myocytes, the rate of development of osmotic fragility, which temporally precedes breakdown of the sarcolemmal membrane, closely follows the rate of development of ischemic contracture.3,4 In the intact heart, development of ischemic contracture has been associated with massive cell disruption. It may contribute to the wave-front pattern of the progression of necrosis by increasing mechanical stress on adjacent myocytes through the intercalated disks.5 In addition, increased wall tension generated by ischemic contracture has been shown to constrict the microvasculature in the ischemic region and to alter the normal transmural distribution of nutritive perfusion,6 exacerbating ischemia and accelerating damage to the endocardium.

Despite these findings, the role of ischemic contracture in the development of irreversible damage remains controversial. The time courses of development of ischemic contracture and of irreversible cell damage closely parallel the time course of myocardial high-energy phosphate depletion,7 known to be a major determinant of loss of viability. We8 and others9-11 have recently shown that myocardial viability (ie, functional recovery with reperfusion) parallels preservation of glycolytic flux during the antecedent interval of ischemia. Maintenance of high glycolytic rates may benefit the ischemic myocardium by preventing ischemic contracture. Preservation of high-energy phosphate stores, protection of sarcolemma by ATP generated in the cytosol, and improved transmural perfusion are additional possible mechanisms.

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The present study was designed to define the relative role of mechanical effects of ischemic contracture and of preservation of high-energy phosphate stores by enhancing glycolysis in the development of myocardial ischemic injury. We used 2,3-butanedione monoxime (BDM), an agent that disrupts excitation-contraction coupling, to attenuate the development of ischemic contracture.\textsuperscript{12,13} The effects of inhibiting ischemic contracture with BDM on membrane integrity and functional recovery during reperfusion were characterized in isovolumically contracting isolated rabbit hearts subjected to global low-flow ischemia. The relative contributions of the effects of increased intracavitary pressure and of myocardial energy metabolism were evaluated separately either by venting the left ventricle to avoid transmission of pressure to the endocardium through the left ventricular balloon or by enhancing myocardial ATP production by stimulating the glycolytic pathway with supraphysiological concentrations of glucose and insulin.

**Materials and Methods**

**Isolated Hearts**

Hearts were removed from 1.5- to 2.0-kg New Zealand White rabbits and perfused retrogradely at 37°C through the aorta as previously described.\textsuperscript{14} Hearts were paced at 180 beats per minute and perfused with nonrecirculating modified Krebs-Henseleit buffer containing physiological concentrations of bovine serum albumin (0.4 mmol/L, United States Biochemical Corp), palmitate (0.4 mmol/L), glucose (5 mmol/L), and insulin (70 μU/L, a physiological level) and the following ions: (mEq/L): sodium 142, chloride 123, potassium 5.8, calcium 2.0, magnesium 1.2, sulfate 1.2, bicarbonate 24.9, and phosphate 1.4. The perfusate was equilibrated with a mixture of 95% O₂/5% CO₂ to maintain perfusate P O₂ at >600 mm Hg.

Left ventricular pressure was measured with a compliant latex balloon placed in the left ventricular cavity via the left atrium and connected to a P23Db pressure gauge transducer (Gould Laboratories). The first derivative of left ventricular pressure (left ventricular dp/dt) was obtained by electronic differentiation of the pressure signal. Heart rate, left ventricular pressure, left ventricular dp/dt, and coronary perfusion pressure were recorded continuously on a strip-chart recorder.

**Experimental Protocol**

After initial isolation and surgical preparation, hearts were perfused at constant flow (18 to 20 mL/min) and allowed to equilibrate for 55 minutes. Left ventricular end-diastolic pressure was initially set at 4 to 6 mm Hg by adjusting the volume of the left ventricular balloon. Once a stable level was attained, no further volume changes were made. All hearts were perfused with buffer containing glucose as the only energy-providing substrate for the first 25 minutes of the equilibration period and with both glucose and palmitate thereafter. To induce ischemia, flow was reduced to 10% of baseline (1.8 to 2.0 mL/min) for 60 or 120 minutes. To ensure tissue hypoxia during ischemia, the perfusate was equilibrated with room air and CO₂ adjusted to maintain the pH at 7.40. After ischemia, flow and oxygenation were restored to preischemic levels over a 3-minute period, and perfusion was continued for another 45 minutes.

The role of ischemic contracture in the pathogenesis of irreversible injury during and after 60 minutes of low-flow ischemia was evaluated in three groups of hearts. In the control group (control-60, n = 20), the volume of the left ventricular balloon was held constant throughout the experiment so that changes in left ventricular end-diastolic pressure occurring as a consequence of changes in left ventricular distensibility would be transmitted to the endocardium via the left ventricular balloon and allowed to interfere with endocardial perfusion. In a second group of hearts (isotonic, n = 12), the volume of the left ventricular balloon was constantly adjusted throughout ischemia and reperfusion so that left ventricular end-diastolic pressure never exceeded its preischemic values. In this group, contracture was thus allowed to develop, but the transmission of pressure to the endocardium through an increase in left ventricular cavity pressure was precluded. In a third group of hearts (BDM-60, n = 12), ischemic contracture was prevented by administration of 20 mmol/L BDM (Sigma Chemical Co), which was introduced into the perfusate at the time of ischemia and continued for the duration of the ischemic period. Pilot studies had shown that 20 mmol/L BDM resulted in complete cessation of contractile activity that was totally reversible on withdrawal of the drug. In each experiment, the Ca\(^{2+}\)-chelating properties of BDM\textsuperscript{15} were overcome by increasing the perfusate concentration of Ca\(^{2+}\) to 3 mM/L in the perfusate (ie, free Ca\(^{2+}\) content was equal). To ensure that the effects of BDM were not due to the change in osmolality associated with its addition, the osmolality of the perfusate was raised in control hearts with the addition of 20 mmol/L sucrose.

Because initial results indicated that BDM not only prevented the development of ischemic contracture but also reduced the rate of depletion of ATP with ischemia (presumably by decreasing oxygen demand), additional experiments were conducted in which the duration of ischemia was increased from 60 to 120 minutes. Results from 12 isovolumic control hearts (control-120) were compared with those obtained from an equal number of hearts treated with 20 mmol/L BDM during this prolonged period of ischemia (BDM-120). The influence of enhanced glycolysis was characterized in an additional group of hearts treated with BDM by doubling the concentration of both glucose and insulin (BDM-120 and double glucose/insulin, n = 12) in the perfusate.

After completion of each study, hearts were rapidly removed from the perfusion apparatus and blotted, and the left ventricle was weighed. Tissue water content was determined by reweighing the left ventricle after drying to constant weight.

**Collection and Analysis of the Samples**

Coronary venous effluent was collected through the pulmonary artery, and P O₂, P CO₂, and pH were monitored regularly by means of a pH-blood gas analyzer (model IL 213, Instrumentation Laboratories). Myocardial oxygen consumption (MVO₂) was calculated as MVO₂= [(0.003×P O₂ arterial)−(0.003×P O₂ venous)]×total flow/ventricular weight. Glucose and lactate concentrations were determined in samples of aortic perfusate and pulmonary effluent by use of enzymatic methods.\textsuperscript{16,17} Glucose extraction and lactate production were calculated from the measured arterial (aortic)−pulmonary arterial effluent differences multiplied by flow divided by left ventricular weight and expressed in micromoles per gram left ventricular dry weight per minute. Creatine kinase activity was measured spectrophotometrically in samples obtained from the pulmonary artery effluent.\textsuperscript{18} At selected time intervals during ischemia, samples of the pulmonary effluent were assayed for K\(^+\) with a K\(^+\)-sensitive electrode (Nova Biochemical).

**Tissue Metabolic Assays**

After careful removal of the atria and the right ventricular free wall, six hearts from each group were rapidly frozen at the end of ischemic period (while still being perfused) with Wollenberger clamps cooled with liquid nitrogen. The hearts were then weighed and stored at -70°C until assay.

**High-Energy Phosphate Content**

Tissue high-energy phosphate content was determined by high-pressure liquid chromatography (HPLC) of neutralized
perchlorate extracts of rapidly frozen myocardium. For separation of the nucleotides, a model RP-318 Bio-Rad Hi-Pore reverse-phase column (250×4.6 mm, Bio-Rad Laboratories) was used with a programmed mobile phase solvent system consisting of 0.1 mol/L HPLC-grade monobasic ammonium phosphate in deionized water for 10 minutes, which was switched to 0.1 mol/L monobasic ammonium phosphate in 10% methanol for 10 minutes, followed by a return to 0.1 mol/L monobasic ammonium phosphate in deionized water for an additional 10 minutes. Solvents were adjusted to pH 5.5 with NH₄OH and degassed with helium. Elution was performed at 0.5 mL/min, and a variable-wavelength UV/vis spectrophotometer (model 757, Kratos Analytical Instruments) at 214 nm was used for detection of phosphocreatine (PCR), creatine, AMP, ADP, and ATP. All nucleotides were quantified by integration of peaks of unknowns to integration of peaks of known concentrations of standards. Measurements are expressed as micromoles per gram left ventricular dry weight.

**Tissue Lactate and Glycogen Content**

Myocardial tissue lactate was determined spectrophotometrically on neutralized perchlorate extracts of frozen myocardium. Measurements are expressed as micromoles per gram left ventricular dry weight. Glycogen levels were measured by use of an enzymatic assay. Measurements are expressed as micromoles of C₆ equivalents per gram left ventricular dry weight.

**Measurement of Perfusion Area**

Development of ischemic contracture may cause a nonuniform distribution of flow. To define the relative contribution of this phenomenon to myocardial ischemic injury, the perfusable region was delineated in three hearts of each group subjected to 60 minutes of low-flow ischemia by perfusing the hearts with 2% lissamine green added to the ischemic perfusate during the last 5 minutes of ischemia. Hearts were then removed from the perfusion apparatus and blotted, the right ventricular free wall was removed, and the left ventricle was fixed with 10% formalin. After a minimum of 48 hours, the hearts were sliced into serial 1-mm-thick cross sections parallel to the atrioventricular groove. The regions that had been perfused were dark green. Those that were not remained pale. The areas were traced onto transparent film, and the area of perfusion was quantified by planimetry and expressed as a percentage of the total left ventricular cross-sectional area.

**Statistical Analysis**

Values are expressed as mean±1 SD. Significance of differences was determined with a one-way ANOVA for repeated measurements, with additional post hoc tests for differences (Bonferroni formulation). The relations between left ventricular functional and metabolic parameters were assessed by linear regression analysis or nonlinear least-squares fitting routines where appropriate. Values of P<.05 were considered to be indicative of statistically significant differences.

**Results**

**Effects of BDM and Ventricular Venting on Myocardial Function and Metabolism in Hearts Subjected to 60 Minutes of Low-Flow Ischemia**

**Mechanical Performance**

After 55 minutes of equilibration, baseline left ventricular developed pressure (systolic minus end-diastolic) was similar among the three groups (Table 1, Fig 1). Within 1 minute after the onset of ischemia, hearts treated with BDM showed no residual contractile activity. In contrast, most hearts in the two other groups retained some degree of contractile function (although function was severely impaired with a developed pressure typically <10 mm Hg) throughout ischemia.

Ischemic contracture was defined as a rise of 4 mm Hg or more in left ventricular end-diastolic pressure after the onset of ischemia. In control hearts, in which the volume of the left ventricular balloon was held constant throughout the experiment, ischemic contracture developed 11±10 minutes after the onset of ischemia and reached a peak contracture pressure of 48±14 mm Hg after 60 minutes of ischemic perfusion (Fig 1). In hearts in which the increase in resting tension was offset by deflation of the left ventricular balloon to maintain end-diastolic pressure constant (isotonic group), balloon deflation needed to be initiated 11±4 minutes after the onset of ischemia. By 1 hour, the volume of the left ventricular balloon had been reduced by 48±13%. Hearts treated with BDM exhibited a minimal increase in left ventricular end-diastolic pressure during ischemia (Fig 1).

With reperfusion, left ventricular end-diastolic pressure continued to increase in control hearts (to 58±30 mm Hg). Similarly, in hearts from the isotonic group, the left ventricular balloon had to be deflated further, by 57±14% of its initial volume, to maintain end-diastolic pressure at baseline levels. In contrast, in hearts treated with BDM, only a moderate increase in left ventricular end-diastolic pressure was observed during reperfusion (to a maximum of 20±7 mm Hg, P<.01 compared with control hearts, Fig 1). After 45 minutes of reperfusion, recovery of developed pressure averaged 51±23 mm Hg (56±23% of baseline) in the control group, 59±9 mm Hg (62±7% of baseline) in the isotonic group (P=NS compared with the control group), and 76±6 mm Hg (86±10% of baseline) in hearts treated with BDM (P<.01 compared with the control group, P<.05 compared with the isotonic group) (Fig 1).

**Myocardial Energy Metabolism During Ischemia**

**MVO₂** With ischemia, MVO₂ averaged 0.44±0.09 μmol/g dry weight per minute and did not differ among the groups (Table 1).

**Glycolytic flux.** Glycolytic flux was assessed by measuring glucose uptake, lactate production, and glycogen depletion. During the equilibration interval, myocardial glucose uptake averaged 8.6±7.2 μmol/g dry weight per minute, and myocardial lactate production was 6.5±3.3 μmol/g dry weight per minute and did not differ among the groups. With the onset of ischemia, both glucose uptake and lactate production increased significantly in all groups. The increase was, however, blunted in hearts treated with BDM. Glucose uptake and lactate production subsequently declined in all three groups of hearts (Table 1). Hearts subjected to treatment with BDM maintained a higher level of lactate production compared with hearts in the control group (Table 1).

Preischemic tissue glycogen averaged 75±10 μmol C₆ equivalents per gram dry weight. As shown in Table 2, after 60 minutes of ischemia, tissue glycogen was markedly reduced in hearts in the control and isotonic groups. It was better preserved in hearts treated with BDM. Tissue lactate content was significantly higher in control hearts than in hearts from...
### TABLE 1. Selected Hemodynamic and Metabolic Values From Isolated Rabbit Hearts

<table>
<thead>
<tr>
<th>Group</th>
<th>Observation Period</th>
<th>Ischemia, min</th>
<th>Reperfusion</th>
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<tbody>
<tr>
<td></td>
<td>Equilibration</td>
<td>5</td>
<td>60</td>
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<tr>
<td>60-Min ischemia</td>
<td></td>
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<td></td>
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<tr>
<td>Control-60</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LVDP, mm Hg</td>
<td>92±9</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>9±3</td>
<td>9±5</td>
<td>48±14</td>
</tr>
<tr>
<td>M(\text{Vo}_2), (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>26.5±3.0</td>
<td>0.48±0.09</td>
<td>0.48±0.09</td>
</tr>
<tr>
<td>MGU, (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>6.8±11.3</td>
<td>4.8±1.4</td>
<td>1.8±0.5</td>
</tr>
<tr>
<td>MLP, (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>7.3±2.7</td>
<td>10.9±2.1</td>
<td>2.7±1.1</td>
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<tr>
<td>Isotonic</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LVDP, mm Hg</td>
<td>97±6</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>7±2</td>
<td>...</td>
<td>...</td>
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<tr>
<td>M(\text{Vo}_2), (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>25.1±4.2</td>
<td>0.44±0.11</td>
<td>0.41±0.07</td>
</tr>
<tr>
<td>MGU, (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>6.5±4.6</td>
<td>4.4±1.9</td>
<td>1.8±0.5</td>
</tr>
<tr>
<td>MLP, (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>4.9±2.7</td>
<td>9.5±1.4</td>
<td>3.7±0.5</td>
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<tr>
<td>BDM-60</td>
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<tr>
<td>LVDP, mm Hg</td>
<td>90±7</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>7±2</td>
<td>7±2</td>
<td>12±3*</td>
</tr>
<tr>
<td>M(\text{Vo}_2), (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>22.3±3.9</td>
<td>0.40±0.08</td>
<td>0.40±0.08</td>
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<tr>
<td>MGU, (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>10.8±6.1</td>
<td>3.5±0.5</td>
<td>2.2±1.0</td>
</tr>
<tr>
<td>MLP, (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>7.8±3.6</td>
<td>8.4±1.8</td>
<td>4.7±1.4*</td>
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<tr>
<td>120-min ischemia</td>
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<td>Control-120</td>
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<td></td>
<td></td>
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<tr>
<td>LVDP, mm Hg</td>
<td>91±8</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>7±2</td>
<td>7±2</td>
<td>48±14</td>
</tr>
<tr>
<td>M(\text{Vo}_2), (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>26.5±2.7</td>
<td>0.52±0.07</td>
<td>0.50±0.11</td>
</tr>
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<td>MGU, (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>15.9±4.9</td>
<td>5.2±1.0</td>
<td>2.4±1.0</td>
</tr>
<tr>
<td>MLP, (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>12.3±4.7</td>
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<tr>
<td>BDM-120</td>
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<tr>
<td>LVDP, mm Hg</td>
<td>96±2</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>6±2</td>
<td>6±3</td>
<td>9±4*</td>
</tr>
<tr>
<td>M(\text{Vo}_2), (\mu\text{mol} \cdot \text{min}^{-1})</td>
<td>25.1±4.3</td>
<td>0.47±0.12</td>
<td>0.48±0.13</td>
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<tr>
<td>MGU, (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>10.2±5.8</td>
<td>4.1±1.4</td>
<td>2.8±1.0</td>
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<tr>
<td>MLP, (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>9.2±5.1</td>
<td>8.4±2.2*</td>
<td>4.7±1.3\§</td>
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<tr>
<td>BDM-120 and double glucose/insulin</td>
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<tr>
<td>LVDP, mm Hg</td>
<td>94±7</td>
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<td>...</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>6±3</td>
<td>6±4</td>
<td>6±4</td>
</tr>
<tr>
<td>M(\text{Vo}_2), (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>27.9±5.9</td>
<td>0.57±0.18</td>
<td>0.60±0.17</td>
</tr>
<tr>
<td>MGU, (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>13.4±10.6</td>
<td>7.0±2.4\¶</td>
<td>5.1±2.2|</td>
</tr>
<tr>
<td>MLP, (\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})</td>
<td>10.6±4.9</td>
<td>11.2±2.5*</td>
<td>6.5±1.4*</td>
</tr>
</tbody>
</table>

LVDP indicates left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; M\(\text{Vo}_2\), myocardial oxygen consumption; MGU, myocardial glucose uptake; MLP, myocardial lactate production; control-60, control group subjected to 60 minutes of ischemia; isotonic, group subjected to 60 minutes of ischemia in which volume was adjusted so that left ventricular end-diastolic pressure never exceeded its preischemic value; BDM-60, group treated with 2,3-butanedione monoxime (BDM) and subjected to 60 minutes of ischemia; control-120, control group subjected to 120 minutes of ischemia; BDM-120, group treated with BDM and subjected to 120 minutes of ischemia; BDM-120 and double glucose/insulin, group treated with BDM and a doubled concentration of glucose and insulin and subjected to 120 minutes of ischemia. Values are the mean± SD of 12 experiments during equilibration and ischemia (except control-60 [\(n=20\)] and 6 experiments during reperfusion.

\*P<.01 vs corresponding control value; \(\dagger\)P<.05 vs corresponding isotonic value; \#P<.001 and \$P<.05 vs corresponding control value; and ||P≤.01 and \*\*P<.05 vs corresponding BDM-120 value.
distribution of the dye in a representative heart from each group. In these midventricular cross sections, the dark areas correspond to normally perfused myocardium, and the lighter areas correspond to myocardium with decreased perfusion. In control hearts, the extent of the perfusion defect averaged 45±17% of the left ventricle. It averaged only 5±3% in ischemic hearts (P<.01 compared with control hearts) and 2±2% in hearts treated with BDM (P<.001 compared with control hearts, P=NS compared with ischemic hearts). Despite the decrease in the extent of the perfusion deficit in ischemic hearts, the finding of increased tissue lactate content in hearts in the isotonic group compared with those in the BDM-60 group (Table 2) suggests that microscopic "contracture" may have impaired nutritional perfusion in these hearts.

Effects of BDM and Enhanced Glycolysis on Myocardial Function and Metabolism in Hearts Subjected to 120 Minutes of Low-Flow Ischemia

Because the results obtained did not differentiate mechanical as opposed to metabolic protective effects attributable to the prevention of contracture with BDM, experiments were performed in which the duration of ischemia was increased from 60 to 120 minutes. The additional experiments involved three groups of hearts subjected to 120 minutes of low-flow ischemia followed by 45 minutes of reperfusion. Results from 12 control hearts (control-120) were compared with those obtained in 12 hearts treated with 20 mmol/L BDM (BDM-120). Since we had previously demonstrated that stimulation of glycolytic metabolism protected ischemic myocardium, an additional group of 12 hearts was studied in which prevention of ischemic contracture with BDM was combined with stimulation of glycolysis by doubling the perfusate concentration of glucose and insulin (to 10 mmol/L and 140 mU/L, respectively) during the interval of ischemia (BDM-120 and double glucose/insulin).

Mechanical Performance

After 55 minutes of equilibration, baseline left ventricular developed pressure was similar among the three groups of hearts (Table 1). With the onset of ischemia, systolic function deteriorated rapidly in all groups (Fig 4).

In control hearts, ischemic contracture developed 10±5 minutes after the onset of ischemia. End-diastolic pressure peaked at 53±9 mm Hg after 90 minutes of ischemia. In hearts treated with BDM, the increase in left ventricular end-diastolic pressure was markedly attenuated (ie, contracture was prevented) to only 10±6 mm Hg in hearts exposed to BDM (P<.001 compared with control-120 hearts) and to 7±4 mm Hg in hearts exposed to BDM and double glucose/insulin (P<.001 compared with control-120 hearts, P=NS compared with BDM-120 hearts).

With reperfusion, left ventricular end-diastolic pressure plateaued in control hearts at ≈55 mm Hg. It increased modestly to 34±9 mm Hg in hearts treated with BDM (P<.01 compared with control-120 hearts) and to 19±7 mm Hg in hearts receiving BDM and double glucose/insulin (P<.0001 compared with control-120 hearts, P<.05 compared with BDM-120 hearts) (Fig 4). After 45 minutes of reperfusion, developed pressure recovered to only 37±4 mm Hg (41±4% of
TABLE 2. Effects of Low-Flow Ischemia on Selected Myocardial High-Energy Phosphate, Lactate, and Glycogen Content

<table>
<thead>
<tr>
<th></th>
<th>PCr, μmol/g LV dry wt</th>
<th>ATP, μmol/g LV dry wt</th>
<th>ADP, μmol/g LV dry wt</th>
<th>AMP, μmol/g LV dry wt</th>
<th>TAN, μmol/g LV dry wt</th>
<th>Lactate, μmol/g LV dry wt</th>
<th>Glycogen, μmol C₆ equivalents/g dry wt</th>
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<tbody>
<tr>
<td>Equilibration</td>
<td>53±6</td>
<td>25.8±1.7</td>
<td>7.0±0.7</td>
<td>1.0±0.3</td>
<td>33.7±1.9</td>
<td>21±12</td>
<td>75±10</td>
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<tr>
<td>60-min ischemia</td>
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<tr>
<td>Control-60</td>
<td>10±2</td>
<td>6.3±1.4</td>
<td>4.2±0.3</td>
<td>4.0±0.7</td>
<td>12.1±6.0</td>
<td>96±13</td>
<td>14±5</td>
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<tr>
<td>Isotonic</td>
<td>13±2</td>
<td>8.0±1.9</td>
<td>3.7±0.2</td>
<td>2.2±0.7*</td>
<td>13.9±1.1</td>
<td>66±15†</td>
<td>18±6</td>
</tr>
<tr>
<td>BDM-60</td>
<td>37±8*</td>
<td>16.5±2.3*</td>
<td>4.5±0.8</td>
<td>1.7±0.4*</td>
<td>22.6±3.4*</td>
<td>34±11§</td>
<td>58±21*†</td>
</tr>
<tr>
<td>120-min ischemia</td>
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</tr>
<tr>
<td>Control-120</td>
<td>12±4</td>
<td>4.5±1.8</td>
<td>3.0±0.5</td>
<td>3.4±0.5</td>
<td>10.8±1.9</td>
<td>120±29</td>
<td>13±6</td>
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<tr>
<td>BDM-120</td>
<td>35±8</td>
<td></td>
<td></td>
<td>15.6±3.8</td>
<td>4.8±0.7*</td>
<td>2.0±0.6†</td>
<td>22.5±4.3†</td>
</tr>
<tr>
<td>BDM-120 and double glucose/insulin</td>
<td>49±5</td>
<td></td>
<td>20.9±3.9[#]</td>
<td>5.7±1.3</td>
<td></td>
<td>2.0±0.7</td>
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PCr indicates creatine phosphate; LV, left ventricular; TAN, total adenine nucleotide content; control-60, control group subjected to 60 minutes of ischemia; isotonic, group subjected to 60 minutes of ischemia in which volume was adjusted so that left ventricular end-diastolic pressure never exceeded its preischemic value; BDM-60, group treated with 2,3-butanedione monoxime (BDM) and subjected to 60 minutes of ischemia; control-120, control group subjected to 120 minutes of ischemia; BDM-120, group treated with BDM and subjected to 120 minutes of ischemia; and BDM-120 and double glucose/insulin, group treated with BDM and a doubled concentration of glucose and insulin and subjected to 120 minutes of ischemia. Values are the mean±1 SD of five or six experiments.

*P<.001 and †P<.01 vs control-60; ‡P<.001 and §P<.01 vs isotonic; ||P<.001 and ††P<.01 vs control-120; and ###P<.01 vs BDM-120.

Fig 2. Bar graph showing the cumulative amount of creatine kinase released by hearts during 45 minutes of reperfusion. With 60 minutes of ischemia followed by reperfusion, either venting the left ventricle or inhibiting contracture with 2,3-butanedione monoxime (BDM) diminished creatine kinase release. With longer periods of ischemia, although creatine kinase release was diminished by inhibition of ischemic contracture, it was almost completely prevented by enhancing glycolysis (see text). Control-60 indicates control group subjected to 60 minutes of ischemia; isotonic, group subjected to 60 minutes of ischemia in which volume was adjusted so that left ventricular end-diastolic pressure never exceeded its preischemic value; BDM-60, group treated with BDM and subjected to 60 minutes of ischemia; control-120, control group subjected to 120 minutes of ischemia; BDM-120, group treated with BDM and subjected to 120 minutes of ischemia; and BDM-120+G10, group treated with BDM and a doubled concentration of glucose and insulin and subjected to 120 minutes of ischemia. *P<.05 compared with control-60; †P<.05 and ‡P<.01 compared with control-120; and ††P<.05 compared with BDM-120.

Baseline (80±7 mm Hg, 88±7% of baseline) in control hearts and to 64±10 mm Hg (68±7% of baseline) in hearts receiving BDM (P<0.001 compared with control-120 hearts). In contrast, developed pressure was preserved to nearly baseline levels (80±7 mm Hg, 88±7% of baseline) in hearts receiving BDM and double glucose/insulin (P<0.001 compared with control-120 hearts, P<0.01 compared with BDM-120 hearts).

Myocardial Energy Metabolism During Ischemia

MV02. With ischemia, MV02 averaged 0.53±0.15 μmol/g dry weight per minute and did not differ between groups (Table 1).

Glycolytic flux. During the equilibration interval, myocardial glucose uptake averaged 13.2±7.7 μmol/g dry weight per minute, and myocardial lactate production was 10.7±4.9 μmol/g dry weight per minute and did not differ between the groups. With the onset of ischemia, both glucose uptake and lactate production increased significantly in all groups, although the increase was blunted in hearts treated with BDM. Glucose uptake and lactate production subsequently declined in all groups (Fig 5). However, hearts treated with BDM and double glucose/insulin maintained significantly higher lactate output throughout ischemia (to 6.7±1.3 μmol/g dry weight per minute after 120 minutes of ischemia, 60% of initial rates), whereas hearts treated with BDM and the control perfusate concentration of glucose exhibited a more pronounced decrease in lactate release over time (to 4.0±0.9 μmol/g dry weight per minute after 120 minutes of ischemia, 47% of initial rates). Inhibition of glycolysis was particularly severe in control hearts. Residual lactate efflux averaged 2.4±0.8 μmol/g dry weight per minute (17% of initial rates) after 120 minutes of ischemia. Total lactate production with ischemia (lactate release and tissue lactate) was significantly greater in hearts treated with BDM and double
glucose/insulin (872 ± 158 μmol/g dry weight) compared with that in the two other groups of hearts (709 ± 87 and 619 ± 127 μmol/g dry weight in control-120 and BDM-120 hearts, respectively; \( P < .01 \) for each compared with BDM-120 and double glucose/insulin hearts).

Changes in lactate release were paralleled by directional changes in exogenous glucose uptake (Fig 5). In hearts treated with BDM and glucose/insulin, lactate production resulted almost exclusively from metabolism of exogenous glucose with no significant contribution of glycogen breakdown. As shown in Table 2, after 120 minutes of ischemia, tissue glycogen decreased to 70 ± 15 μmol C₆ equivalents per gram dry weight (93% of preischemic levels, \( P = \text{NS} \) compared with the end of equilibration). In contrast, it was significantly reduced in control hearts (to 13 ± 6 μmol C₆ equivalents per gram dry weight, 17% of preischemic levels) and in hearts treated with BDM and control perfusate concentrations of glucose/insulin (to 47 ± 12 μmol C₆ equivalents per gram dry weight, 63% of preischemic levels). Accumulation of tissue lactate was significantly greater in control hearts than in the two groups of hearts treated with BDM (Table 2).

**High-energy phosphates.** After 120 minutes of ischemia, concentrations of PCR and ATP were markedly decreased in control hearts. Total ATP was well preserved in both groups of hearts treated with BDM, although hearts exposed to double glucose/insulin exhibited significantly greater ATP content than those exposed to only control amounts of glucose/insulin during the interval of ischemia. Total PCR content was not significantly different from baseline in hearts treated with BDM and double glucose/insulin (Table 2).

**Creatine Kinase Release During Ischemia and Reperfusion**

By the end of the interval of ischemia, the rate of creatine kinase release was significantly greater in control hearts (0.26 ± 0.15 U/g dry weight per minute) than in the two groups of hearts treated with BDM (0.15 ± 0.07 U/g dry weight per minute in BDM-120 hearts, \( P < .01 \) compared with control-120 hearts; 0.16 ± 0.05 U/g dry weight per minute in BDM-120 hearts treated with 10 mmol/L glucose, \( P < .05 \) compared with control-120 hearts). After 45 minutes of reperfusion, cumulative creatine kinase release averaged 1011 ± 336 U/g dry weight in control hearts but was reduced to 483 ± 254 U/g dry weight in hearts treated
with BDM alone (P<.05 compared with control-120 hearts). It was reduced even further, to 114±72 U/g dry weight, in hearts treated with BDM and double glucose/insulin (P<.01 compared with control-120 hearts, P<.05 compared with BDM-120 hearts) (Fig 2).

**K⁺ Efflux**

Efflux of K⁺ from the myocardium was used as an additional measure of cell membrane function and integrity and was evaluated by measuring effluent and perfusate K⁺ concentrations at selected intervals. As illustrated in Fig 5, the time course of K⁺ efflux with ischemia was biphasic in all three groups. Shortly after the onset of ischemia, the concentration of K⁺ in the pulmonary artery effluent increased rapidly and similarly in all groups. After a transient reduction, K⁺ efflux increased again in control hearts to 956±346 nEq/g dry weight per minute after 120 minutes of ischemia. This phase was markedly attenuated in hearts treated with BDM (each P<.001 compared with control-120 hearts). However, hearts exposed to BDM with control perfusate levels of glucose/insulin exhibited significantly greater K⁺ efflux (562±249 nEq/g dry weight per minute) than hearts receiving BDM and double glucose/insulin (239±227 nEq/g dry weight per minute, P<.05 compared with BDM-120 hearts). End-ischemic K⁺ efflux correlated significantly with end-ischemic myocardial lactate production (r = -.70, P<.001) (Fig 6).

**The Relation Between Myocardial Injury, Ischemic Contracture, and Energy Metabolism During Ischemia**

Functional recovery (expressed as percent recovery of developed pressure) correlated with end-ischemic tissue ATP (r = .93, P<.01) and with end-ischemic myocardial lactate production (r = .95, P<.001) (Fig 7). Functional recovery also correlated with left ventricular end-diastolic pressure at the end of ischemia (r = -.72, P<.05) (data not shown). Cumulative creatine kinase released (an index of loss of sarcomembran functional integrity and irreversible ischemic injury) correlated inversely with end-ischemic myocardial lactate production (r = -.84, P<.01), with end-ischemic tissue ATP (r = -.76, P<.05), and directly with left ventricular end-diastolic pressure at the end of the interval of ischemia (r = .72, P<.05) (data not shown).

**Fig 5.** Graphs showing the time course of lactate production (top left), potassium efflux (top right), and myocardial glucose uptake (bottom left) during ischemia in control hearts (●) and in hearts treated with 20 mmol/L 2,3-butanedione monoxime (BDM) and either control (●) or double levels (▲) of glucose/insulin. The first point presented represents data obtained at 1 minute of ischemia. Hearts exposed to double concentrations of glucose/insulin exhibited maintenance of lactate production (indicative of maintained anaerobic glycolysis), enhanced glucose uptake, and preserved membrane function (decreased potassium efflux). *P<.05, **P<.01, and ***P<.001 compared with control hearts; #P<.05 and ##P<.01 compared with hearts treated with BDM and 120 minutes of ischemia.

**Fig 6.** Graph showing the relation between myocardial lactate production and potassium efflux at the end of the interval of ischemia in hearts subjected to 120 minutes of low-flow ischemia. The severity of K⁺ loss was inversely related to the preservation of glycolytic flux estimated from myocardial lactate production.
dote to organophosphorus poisoning. BDM has been shown to inhibit myocyte contraction in a dose-dependent manner and to prevent the development of reoxygenation contracture in both isolated cells and intact hearts. Its primary site of action appears to be at the level of the myofilament, since it decreases the sensitivity of the contractile apparatus to calcium and inhibits the ATPase activity of myosin.

In the present study, administration of BDM prevented the development of ischemic contracture, reduced membrane damage, and improved the recovery of contractile function with reperfusion. Although BDM may have protected ischemic myocardium by preventing ischemic contracture and the resultant mechanical stress, BDM administration also reduced the rate of ATP depletion during ischemia. Therefore, it may have exerted protection by reducing metabolic needs and thus maintaining tissue ATP content.

To distinguish between these two possibilities, we extended the period of ischemia from 60 to 120 minutes. After 120 minutes of ischemia, BDM still prevented ischemic contracture and preserved ATP stores, but it did not prevent membrane damage or contractile failure with reperfusion. In contrast, increasing the rate of anaerobic energy production during ischemia in the presence of BDM greatly reduced membrane damage and resulted in almost complete recovery of contractile performance with reperfusion. These observations suggest that energy depletion, particularly insufficient energy production via anaerobic glycolysis, is the main contributor to irreversible ischemic damage and that contracture and its resultant mechanical effects play only a minor role.

**Contribution of Vascular Compression to Myocardial Ischemic Injury**

Acute reduction in coronary blood flow results in the development of ischemic contracture within 5 to 15 minutes. If the ventricular cavity is made incompressible by insertion of a noncompressible balloon, as was the case in our control hearts, then ischemic contracture is manifested by increased cavity pressure and intramural wall tension—changes that can result in constriction of arterioles and capillaries, particularly in the endocardium, where wall stress is greatest. Development of contracture could thus lead to a progressive reduction in nutritive perfusion, thereby exacerbating ischemia, and to insufficient washout of potentially toxic metabolites, such as lactate or hydrogen ions, thought to contribute directly to ischemic injury. Such an increase in resting tension could attenuate the extent of reperfusion with the resumption of flow, contribute to the no-reflow phenomenon, and consequently limit the recovery of contractile function.

The present observations confirm that large regions of the endocardium lose their capacity to accommodate reperfusion and accumulate tissue lactate when ischemic contracture develops in the presence of an incompressible intraventricular balloon. The data are consistent with previous observations that regions of decreased perfusion at reflow are confined to those within the region of ultimate necrosis and that progressive loss of “perfusability” only occurs in regions of the myocardium where myocytes are already in contracture and have presumably become nonviable. The se-
verity of the deficiency of perfusion and the accumulation of lactate were considerably mollified when the intraventricular balloon was deflated during the course of ischemia to maintain left ventricular end-diastolic pressure constant. Nevertheless, ventricular venting still resulted in increased tissue lactate content (likely indicative of impaired microvascular perfusion) and decreased high-energy phosphate stores and did not result in improved functional recovery with subsequent reperfusion.

**Role of Ischemic Contracture and Increased Mechanical Stress**

Creatine kinase release, a measure of membrane damage,\(^3\)\(^,\)\(^3\)\(^2\) was greatest in control hearts. More striking damage with ischemic contracture under isometric conditions has been described previously and has been attributed to the direct effect of mechanical stress.\(^6\) The fact that creatine kinase release was diminished in vented hearts supports the contention that the severity of membrane damage is at least partially the result of mechanical stress imposed by contracture. Such mechanical stress may contribute to ischemic damage, but its prevention does not result in improved functional recovery with reperfusion. Therefore, it is unlikely that the beneficial effects afforded by BDM in the present study were related only to the prevention of contracture.

**Contribution of Myocardial Energy Depletion to Irreversible Injury**

The role of high-energy phosphate depletion in the development of irreversible damage has been long debated.\(^1\)\(^,\)\(^2\) Because ATP is involved in diverse processes critical to cell viability, it seems reasonable that ATP depletion could cause lethal injury simply by disrupting these processes. Previous results have indicated that ATP and adenine nucleotide depletion correlate closely with the development of irreversible injury with severe ischemia. In the present study, ATP depletion was associated with contractile failure and with creatine kinase release with reperfusion. Despite this relation, the results tend to indicate that total ATP or adenine nucleotide depletion might not be the most important determinant of injury. Extending the duration of ischemia from 60 to 120 minutes in hearts treated with BDM and control amounts of glucose and insulin did not lead to further depletion of ATP but resulted in greater creatine kinase release and more functional impairment despite reperfusion. Both were prevented by increasing the rate of anaerobic ATP production (estimated from myocardial lactate production) in a manner consistent with the hypothesis that the turnover rate of high-energy phosphate is a more critical determinant of survival of myocardium than simply the gross tissue concentration of ATP. This interpretation is supported by the large disparity between total cellular ATP reserves (=25 μmol/g dry weight) and the amount of ATP generated via anaerobic glycolysis during the interval of ischemia (709±87 μmol/g dry weight in control hearts, 619±127 μg/g dry weight in hearts treated with BDM alone, and 872±158 μmol/g dry weight in hearts treated with BDM and double glucose/insulin; \(P<.01\) between BDM-120 and high glucose/insulin hearts and either control-120 or BDM-120 hearts). Therefore, it is not surprising that viability correlates more closely with cumulative anaerobic ATP production than with total cellular ATP content.

**Compartmentation of ATP Produced by Glycolysis**

Glycolysis may be a preferential source of high-energy phosphates supporting membrane function and preventing irreversible damage induced by ischemia.\(^3\)\(^3\)\(^-\)\(^3\)\(^6\) Compartmentation of ATP may be related to localization of key glycolytic enzymes at specific sites in the cell that maintain the local concentrations of adenine nucleotides independent of total tissue ATP. Glycolysis may be the primary source of ATP supporting the activity of Na\(^+\),K\(^+\)-ATPase, an enzyme critical to the maintenance of cell volume and electrolyte homeostasis. In the present study, increasing the rate of anaerobic ATP production by increasing the concentrations of glucose and insulin in the perfusate was associated with a significant decrease in the loss of K\(^+\) from ischemic myocardium. Although most of the K\(^+\) loss in control hearts appeared to be attributable to cell membrane disruption (because it was associated with loss of high molecular weight molecules like creatine kinase), the differences in K\(^+\) loss between the two groups of hearts treated with BDM may reflect more complete membrane protection in hearts with higher glycolytic rates.\(^3\)\(^3\)\(^-\)\(^3\)\(^6\)

Alternatively, glycolytic ATP production could favorably influence cellular Ca\(^+\)\(^+\) homeostasis during ischemia. Both glycolenolytic and glycolytic enzymes have been associated with the sarcoplasmic reticulum and the sarcolemma.\(^3\)\(^7\) Analysis of isolated membrane vesicles from smooth muscle cells have shown that membrane Ca\(^+\)\(^+\) transport is preferentially supported by glycolysis.\(^3\)\(^8\) Maintenance of high rates of anaerobic glycolysis could thus protect ischemic myocardium by preventing Ca\(^+\)\(^+\) overload\(^3\)\(^9\) during the course of ischemia and hence prevent its deleterious effects on the cytoskeleton and on cell membranes. In addition, prevention of Ca\(^+\)\(^+\) overload may reduce the severity of contractile dysfunction with reperfusion, as judged from the severity of stunning seen with the restoration of flow.\(^4\)\(0\)

It should be emphasized that during ischemia, hearts were exposed to low levels of perfusate flow and perfusate oxygen content. Accordingly, myocardial oxygen delivery and use were quite low (<1% of normal). These conditions would be anticipated to preferentially stimulate anaerobic glycolysis. In vivo, residual myocardial perfusion in areas of ischemia is typically 10% to 30% of normal. Whether the stimulation of anaerobic glycolysis observed in this isolated heart preparation can be extrapolated to the intact animal remains to be determined.

**Clinical Implications**

The beneficial effects of blockade of the contractile machinery with BDM may have important implications for the management of myocardial ischemia in hearts undergoing global ischemia, such as hearts on bypass or those being preserved for transplantation. The results suggest that BDM may be an effective cardioplegic agent. Recent preliminary observations demonstrating a reduction in the severity of myocardial stunning after intracoronary administration of BDM during the course of ischemia\(^4\) further support this point of view. BDM has been shown to protect the myocardium from injury due to dissection\(^4\)\(^2\) and to reduce the severity of reoxy-
generation injury in isolated myocytes,24 in isolated hearts,25 and in intact animals. The present data confirm that BDM can protect the myocardium from ischemic injury and preserve adenine nucleotide content during ischemic periods as long as 120 minutes. However, it seems that its administration is accompanied by a slight increase in membrane fragility, as suggested by the disproportionately high amounts of creatine kinase released from hearts treated with BDM alone despite relatively well-preserved tissue ATP. Although this might reflect only the relative lack of efficacy of total ATP to protect cell membranes, we cannot dismiss the possibility of a direct toxic effect of BDM that can potentially dephosphorylate membrane proteins.43 Our data show that if BDM is administered concomitantly with increased glycolytic substrate, it results in remarkable protection against ischemic and/or reperfusion injury.

Conclusions

Myocardial energy metabolism during ischemia appears to be the most important determinant of ischemic injury. The mechanical effects of ischemic contracture play only a minor role in this process. The results support the thesis that reperfusion-mediated events are largely a consequence of injury occurring during the antecedent ischemic interval and that only prevention of these ischemia-specific events can effectively preclude reperfusion-induced injury. The findings substantiate previous observations8,44 that glycolysis is critical to myocardial viability by providing ATP locally necessary for preservation of the integrity and function of the sarcotema and probably also the sarcoplasmic reticulum. Accordingly, strategies designed to enhance the glycolytic production of ATP in ischemic myocardium may diminish ischemic injury and augment the salutary effects of reperfusion.

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Vanoverschelde et al. Metabolism in Ischemic Injury
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