ABSTRACT

Under normal conditions, the heart is well oxygenated and consumes energy at a high rate. A nearly constant level of metabolites in the cytosol is maintained in myocardial cells via a balance between energy supply and expenditure. This balance is disrupted, however, in the ischemic setting. During ischemia, cardiac oxygen demand exceeds supply, resulting in decreased levels of high-energy phosphates, including phosphocreatine and adenosine triphosphate. In addition, increased free fatty acid levels inhibit glucose oxidation. Fatty acids, which produce less energy per unit of oxygen consumed compared to glucose, are used as fuel, and glucose metabolism is inhibited at the lactate step. Glycolysis in the absence of glucose oxidation increases lactate levels and intracellular acidosis. Prolonged or severe ischemia is associated with irreversible damage to mitochondrial membrane function and myocardial cell death. Mechanisms underlying these changes involve alterations in intracellular ion concentrations and mitochondrial damage. Metabolic changes in the heart during both short and prolonged ischemic conditions highlight the need for interventions aimed at preventing or reversing the cascade of events that result in cardiac cell death. Reperfusion of the ischemic myocardial tissue is beneficial in terms of potential recovery; however, myocytes alive at the time of reperfusion may become injured due to events initiated during reperfusion. Although not fully understood, reperfusion injury is a multifactorial process and affects various aspects of myocardial function. Production of oxygen free radicals and activation of polymorphonuclear leukocytes are hypothesized contributors to myocardial reperfusion injury. Biochemical changes in cardiac metabolism resulting from ischemia as well as consequences of reperfusion are reviewed.


he human heart consumes the energy that supports contraction and relaxation at a high rate. In normal conditions, the heart is well oxygenated and derives its energy primarily from the oxidation of lipids, although it is capable of utilizing glucose and lactate as well. Thus, glycolysis followed by glucose oxidation usually provides about 30% of the energy needs of the heart, while free fatty acids and some lactate supply the remaining 70%. During ischemia, cardiac metabolism is altered. Levels of the high-energy phosphates phosphocreatine and adenosine triphosphate (ATP) fall because energy supply is decreased. Increased levels of fatty acid associated with ischemia inhibit glucose oxidation, resulting in increased lactate and proton levels. In addition, fatty acid metabolism consumes more oxygen per unit of ATP produced than does glucose metabolism. All of these factors lead to an abrupt decrease in contractile activity and, if sufficiently prolonged and severe, decreased high-energy phosphate levels results in loss of ionic homeostasis and irreversible myocardial damage.
During normal perfusion conditions, rates of ATP production and utilization are matched, resulting in the maintenance of a nearly constant level of metabolites in the myocardial cytosol over a range of workloads via an elaborate feedback mechanism. The heart's ability to maintain this equilibrium and sufficient ATP is compromised in the setting of conditions that impair cardiac energy metabolism, such as ischemia.²

**Early Ischemia**

Myocardial ischemia develops when the delivery of oxygenated blood to the myocardium is insufficient to meet the demands required for normal contractile function. During early or mild ischemia, irreversible damage to contractile proteins, mitochondria, and other intracellular organelles does not occur.³ Nonetheless, even early myocardial ischemia can result in significant changes in substrate metabolism.

**Intracellular Acidosis**

Lactate formed via glycolysis during ischemic conditions causes a significant change in cellular energy metabolism.⁴,⁵ Lactate is formed because pyruvate produced by glycolysis is not oxidized in the mitochondria but is reduced to lactate in the cytosol under ischemic conditions. The overall anaerobic glycolytic process results in the formation of lactate plus protons and can be portrayed as: Glucose → 2 Lactate + 2 H⁺. Therefore, lactate production is associated with an acidic intracellular environment that contributes to contractile dysfunction in the ischemic myocardium. In addition, there is decreased ATP production, and accumulation of adenosine diphosphate (ADP), inorganic phosphate, and adenosine (Figure 1).⁴ One mechanism for anginal pain is conversion of ADP to adenosine and resultant stimulation of adenosine A₁ receptors on cardiac afferent sensory neurons.

**Anaerobic Metabolism**

Intracellular acidosis as a consequence of increased glycolysis and decreased glucose oxidation has several additional detrimental effects on the heart's ability to efficiently produce and utilize energy. The cardiomyocyte is no longer able to use energy released from ATP breakdown to perform contractile work and maintain Ca²⁺ homeostasis. In acidic conditions, the calcium force generation relationship is shifted rightwards, resulting in decreased force at a given calcium level. In addition, the amount of ATP required for normal sarcoplasmic Ca²⁺ pump function is increased in an acidic environment. Therefore, during ischemia, relatively more energy generated from ATP is used to regulate Ca²⁺ content in the cytosol and less toward the performance of actual contractile work.⁴

During moderate ischemia, the limited oxygen supply is shifted to support relatively more fatty acid oxidation than glucose oxidation.⁴ Low rates of glucose-derived pyruvate oxidation are due in part to an increase of nicotinamide adenine dinucleotide (NADH) and of the NADH:NAD⁺ ratio in mitochondria.

**Prolonged Ischemia**

Ischemia that is severe or sustained for a prolonged period of time is associated with irreversible damage to mitochondrial membrane function and myocardial cell death.⁶,⁷ Mechanisms underlying these changes involve alterations in intracellular ion homeostasis.

**Ionic Changes**

ATP levels affect several important ion transport pumps in the heart, notably the Na⁺/K⁺ exchange...
and ATP-sensitive K\(^+\) channels.\(^7\)\(^8\) Significant alterations in ion pump activities located in the cellular membrane can also be caused by alterations in membrane phospholipids.

Magnesium has important modulatory functions for ion transport pumps and is involved in the regulation of other intracellular electrolytes, including Ca\(^2+\) and K\(^+\). Mg\(^{2+}\) is not only the most abundant divalent cation in cells but is also a cofactor in more than 300 enzymatic reactions, including phosphotransferases and hydrolases such as ATPases central to energy metabolism.\(^3\) Prolonged ischemia results in decreased levels of intracellular magnesium. This results in a reduction in the activity of the myocyte (Na\(^+\)/K\(^+\)) ATPase. Decreased activity of the Na\(^+\)/K\(^+\) ATPase system leads to an accumulation of sodium in cardiac myocytes. High levels of intracellular sodium support Na\(^+\)/Ca\(^2+\) exchange favoring increased intracellular calcium levels, particularly during reperfusion. Decreased Na\(^+\)/K\(^+\) ATPase activity also decreases intracellular K\(^+\) concentration and may alter repolarization properties.

Intracellular Ca\(^{2+}\) levels also participate in the regulation of many intracellular events important to the maintenance of normal cell function.\(^9\) Intracellular calcium is a main determinant of vascular smooth muscle cell contractility as well as platelet activation and aggregation. Intracellular calcium transients are also related to excitation/contraction coupling. Both short and prolonged ischemia can impair these intracellular calcium functions.

Ischemia alters the transmembrane action potential so as to reduce calcium entry during the plateau phase. Reduced ATP also impairs the ability of sarcoplasmic reticulum to take up calcium from the troponin site. Both of these decrease sarcoplasmic reticulum calcium release at the time of electrical systole. Increased cytosolic calcium during diastole may also raise pressures at that time, further impairing myocardial blood flow, which occurs primarily in diastole. The rise in calcium concentrations associated with ischemia is a powerful mechanism of injury and acts simultaneously on contractile proteins and mitochondria resulting in a deranged cross-talk between them. Intracellular calcium overload is generally accepted as a major mechanism involved in cell death by means of mitochondrial dysfunction. Mitochondrial calcium overload impairs mitochondrial ATP production and is a major cause of mitochondria-mediated cell death.\(^10\)

**ROLE OF MITOCHONDRIA**

Mitochondria occupy a significant volume of cardiomyocytes. During aerobic conditions, mitochondria ATP production via oxidative phosphorylation provides a continuous supply of energy for the contractile activity of the heart.\(^10\)\(^12\) The ischemia-associated increase in mitochondrial calcium has a key role in triggering apoptosis or programmed cell death.\(^13\)\(^14\) Functional and ultrastructural mitochondrial injury occurs early in the course of ischemia and damage progresses as the duration of ischemia lengthens. Following recovery from early ischemia and reperfusion, mitochondrial oxidative function may recover. If ischemia is prolonged, irreversible mitochondrial damage occurs with subsequent myocyte death.

**HIBERNATING AND STUNNED MYOCARDIUM**

As noted above, ischemia occurs when myocardial oxygen supply falls below critical levels. If supply is reduced only modestly, cardiac demand may fall to a level that balances the decreased supply. As supply and demand are still in equilibrium, though at a balanced down-regulated state, no ischemia is present, and necrosis and damage do not occur. This condition, termed “hibernating” myocardium, may persist for long periods of time. Function of hibernating myocardium improves when flow is increased. Hibernating myocardium is a state of chronic contractile dysfunction characterized by dysfunctional but viable myocardium that results from decreased oxygen due to chronic underperfusion.\(^15\)\(^17\) Partial retention of the ability to respond to inotropic challenge is a characteristic of hibernating myocardium. Patients with hibernating myocardium and potentially reversible left ventricular (LV) dysfunction are good candidates for revascularization, although recovery of cardiac function after revascularization may take weeks to months. Characteristics of short- and long-term hibernating myocardium are summarized in Table 1.\(^17\)

Another type of myocardial dysfunction is termed “stunned” myocardium. In this paradigm, flow is restored following an ischemic insult, but function does not return for a prolonged period, at times days or weeks. In this setting, flow is already restored, so proper treatment is support until the stunned myocardium recovers. Stunned myocardium resulting after transient impairment of LV func-
tion during an anginal attack is generally reversible in a few hours or days. In the daily lives of patients with coronary artery disease, LV dysfunction on the basis of stunned myocardium may result from repeated episodes of supply/demand imbalance resulting from transient increases in demand and/or reductions in supply. Hibernating myocardium is characterized by an initial improvement in function with inotropic stimulation, followed by dysfunction due to increased demand-induced ischemia associated with the inotropic response. In stunned myocardium, no metabolic deterioration occurs during inotropic stimulation. Hibernating myocardium exhibits increased glycolytic and decreased tricarboxylic acid (TCA) cycle activity compared with normally perfused myocardium, while stunned myocardium has higher TCA cycle activity relative to a similar workload in normally perfused myocardium.

**RISKS OF REPERFUSION**

Ischemic conditions in the myocardium will result in necrosis if blood flow is not restored within a short time period. Although reperfusion is beneficial in terms of potential recovery, it may also be associated with additional myocardial injury. This event, termed reperfusion injury, can be defined as injury or death of myocytes that were viable at the time of reperfusion as a result of events initiated during the reperfusion period. There are several potential responsible etiologies including calcium overload, free radical production, and inflammatory mediators. A schematic representation of some of these are depicted in Figure 2. This multifactorial process eventually results in a decrease in cardiac performance and cell injury. This section will review some of the factors thought to be associated with myocardial reperfusion injury.

**ANIMAL AND HUMAN DATA**

As noted above, early reperfusion of ischemic myocardium is crucial in preventing irreversible injury and damage. However, reperfusion may itself be associated with processes that injure cardiac muscle as a result of different pathophysiologic consequences.

In vivo and in vitro experiments in animal models have provided most of our current knowledge about the consequences of myocardial reperfusion. The hypothe-

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**Table 1. Characteristics of Short-term and Chronic Hibernating Myocardium**

<table>
<thead>
<tr>
<th>Characteristics of short-term hibernating myocardium:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Balance between the reduced regional myocardial blood flow and the reduced contractile function (perfusion-contraction matching) over several hours</td>
</tr>
<tr>
<td>• Recovery of contractile function during reperfusion</td>
</tr>
<tr>
<td>• Recovery of metabolic parameters (creatine phosphate, free energy change of ATP hydrolysis, lactate)</td>
</tr>
<tr>
<td>• Recruitable inotropic reserve (dobutamine or calcium) at the expense of metabolic recovery</td>
</tr>
</tbody>
</table>

**Characteristics of chronic hibernating myocardium:**

| • Decrease in the number of myofibrils, thin filament complexes, and titin; increase in the number of mitochondria and increased glycogen deposits; interstitial cellular debris; and increased numbers of macrophages, fibroblasts, and collagen; increased apoptosis |
| • Recovery of contractile function during reperfusion |

ATP = adenosine triphosphate.


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**Figure 2. Chain of Events Resulting in Reperfusion Injury**

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ISCHEMIA → Cell death

REPERFUSION → Tissue salvage

PMNs → Vascular Plugging → Autacoids → OFRs → Hydrolyases → O2

"INFLAMMATORY" INJURY

PMNs = polymorphonuclear leukocytes; OFRs = oxygen free radicals.

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sis that reperfusion is associated with injury is supported by studies of animal models evaluating the ability of free-radical scavengers to limit myocardial injury.\textsuperscript{19,22} Although reperfusion-associated alterations are well documented in experimental studies, clinical evidence has not been clearly demonstrated for all aspects (Table 2).\textsuperscript{21} Studies of a similar nature in humans are confounded by the presence and potential influence of a host of clinical factors.\textsuperscript{23} Rather than solely relying on assumptions from animal studies transposed to humans, the utilization of human isolated myocytes, papillary muscle, and atrial myocardium has allowed scientists to more directly investigate the effects and mechanisms of ischemia and reperfusion in humans.

**Mitochondrial Damage**

As noted above, mitochondria have several important functions, including energy production, maintenance of calcium homeostasis, mediation of ischemic and nonischemic preconditioning, and modulation of cellular life and death.\textsuperscript{11} Mitochondria have a small genome that codes for proteins essential in controlling this functional variety. Mitochondrial DNA is highly susceptible to oxidative damage due to the lack of histones, inefficient repair mechanisms, and close proximity to the respiratory chain where reactive oxygen species (ROS) are produced. Oxygen production is increased outside and inside the mitochondria during reperfusion. Consequently, increased production of ROS and a subsequent decrease in antioxidant defenses are observed.

Mitochondria depolarization and subsequent cell death associated with reperfusion are associated with the production of mitochondrial ROS.\textsuperscript{24} Myocardial damage induced by ischemia may be exacerbated during reperfusion by these mitochondrial changes.\textsuperscript{10} Injury to the mitochondrial respiratory chain may be due to oxidative damage, reduction in ATP synthesis, alterations in lipid metabolism, and changes in membrane permeability. The opening of the permeability transition pore, a high-conductance channel in the inner mitochondrial membrane, abolishes mitochondrial ATP production and amplifies the damage by causing the release of NAD\textsuperscript{+}.\textsuperscript{10}

**Calcium Influx**

External stimuli can influence intracellular Ca\textsuperscript{2+} concentrations by regulating various calcium channels.\textsuperscript{25} Myocardial ischemia results in a decrease in ATP production and also a progressive increase in free cytosolic Ca\textsuperscript{2+}. In addition, calcium enters the cell via Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange.\textsuperscript{20} Calcium overload may contribute to reperfusion injury by increasing mitochondrial calcium.\textsuperscript{26} Mitochondrial calcium uptake impairs its ability to generate ATP and limits metabolic recovery. Lastly, calcium-activated proteases may injure or destroy critical intracellular structures resulting in cell death.\textsuperscript{25,26}

**Production of Oxygen Free Radicals**

As previously discussed, ROS production is increased during reperfusion. The significant contribution of ROS to myocardial reperfusion injury is well established.\textsuperscript{27} Overexpression of manganese superoxide dismutase, which decreases superoxide levels, significantly attenuates myocardial necrosis after myocardial ischemia-reperfusion in animal models.

The general hypothesis of ROS-mediated injury during reperfusion is based on the following observations: reperfusion induces impaired myocardial function; ROS are produced during reperfusion; antioxidants diminish myocardial injury; and application of oxidative equivalents induces similar disturbances as seen in vivo during reperfusion.\textsuperscript{28} ROSs are primarily free radicals, ie, they have at least one unpaired electron. Oxygen-derived free radicals pro-

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**Table 2. Potential Effects of Postischemic Reperfusion on the Heart: Available Evidence**

<table>
<thead>
<tr>
<th>Effects of Reperfusion</th>
<th>Experimental Data</th>
<th>Clinical Evidence</th>
<th>Effects of Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen radical generation</td>
<td>+++</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Membrane lipid peroxidation</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Release of oxidized glutathione</td>
<td>+++</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Neutrophil activation</td>
<td>+++</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Contractile impairment</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Electrical instability and arrhythmias</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Myocyte necrosis</td>
<td>+</td>
<td>-</td>
<td>?</td>
</tr>
<tr>
<td>Vascular alteration</td>
<td>+++</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Changes in gene expression</td>
<td>++</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

duced during reperfusion and involved in the pathogenesis of tissue injury include $\text{O}_2^-$, $\text{H}_2\text{O}_2$, and $\text{OH}$. 29 29

One of the most important targets of ROS activity is proteins, including receptors, ionic channels, and components of transduction pathways. Structural changes of these proteins result in altered function and disruption of vital cellular processes.

**POLYMORPHONUCLEAR LEUKOCYTES**

The neutrophil, a major cellular component of the inflammatory response, may represent a key contributor to myocardial reperfusion injury. 22 This theory is supported by experimental data indicating that myocardial injury in response to ischemia and reperfusion is associated with activation of the complement system. Although neutrophils are present in reperfused myocardium, there is uncertainty as to whether they are a cause or merely a consequence of reperfusion injury. 28,30 The latter hypothesis is supported by findings that reperfusion injury may still occur in a neutrophil-free environment and that antineutrophil interventions do not consistently prevent reperfusion injury.

**NECROSIS AND APOPTOSIS**

Necrosis and apoptosis are 2 distinct, yet sometimes overlapping, mechanisms responsible for myocyte death during ischemia and reperfusion. 6,31 Characteristics of apoptosis and necrosis differ and are listed in Table 3. 31 Necrosis is a rapid form of myocyte death capable of triggering a significant inflammatory response. Cells undergoing necrosis are characterized by the loss of cellular membrane integrity, cellular edema, and damaged cytoplasmic organelles. Apoptosis is an energy-dependent, organized, active, and gene-directed process of cellular self-destruction that can be initiated by intracellular genetic programs or second messenger pathways inside the cell following extracellular stimulation. Condensation of the nucleus, fragmentation of the DNA and nucleus, and cell shrinkage are classic signs of cells undergoing apoptosis.

**PRECONDITIONING**

Exposure to a transient, brief period of ischemia results in significant protection during a subsequent prolonged ischemic insult. This protective effect, termed ischemic preconditioning, is associated with a reduction in infarct size, preservation of endothelial function, and attenuation of the neutrophil-mediated inflammatory response. The preconditioning phenomenon has been reproduced in animal models and in organs other than the heart. 32-35 The exact nature of protection observed with preconditioning is not known; however, in early preconditioning, several mediators are released that activate intracellular pathways of signal transduction that activate protein kinase C and other kinases. High levels of extracellular $K^+$ during the preconditioning period results in attenuation of preconditioning's favorable influences on contractile recovery. 36 Apoptosis is also inhibited in ischemic preconditioning models. 6

**CONCLUSIONS**

Heart muscle cannot store oxygen and at any given time is consuming nearly all of the ATP produced. Heart muscle is also, under normal perfusion conditions, capable of quickly adjusting to changes in cardiac workload, and thus maintaining a balance between ATP production and expenditure. During conditions of ischemia, oxygen availability is dimin-

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**Table 3. Characteristics of Apoptosis and Necrosis**

<table>
<thead>
<tr>
<th>Apoptosis</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Requires energy to carry out gene-directed cellular self-destruction</td>
<td>Occurs passively; cell death is caused by severe injury to the cell</td>
</tr>
<tr>
<td>DNA strand cleavage 180-200 base pairs in length</td>
<td>DNA strand cleavage of varying length</td>
</tr>
<tr>
<td>Condensation and fragmentation of the nucleus</td>
<td>Plasma membrane maintains integrity</td>
</tr>
<tr>
<td>Cell shrinkage and blebbing</td>
<td>Cell swelling and lysis</td>
</tr>
<tr>
<td>Phagocytosis of apoptotic bodies by neighboring cells and macrophages prevents release of cellular contents into the extracellular space</td>
<td>Cellular contents are released into the extracellular space</td>
</tr>
<tr>
<td>No inflammatory response</td>
<td>Inflammatory response occurs</td>
</tr>
</tbody>
</table>

ished, resulting in changes in metabolic substrate utilization, contractile dysfunction, ionic shifts, and, ultimately, disruption of intracellular homeostasis and cell death.

Abnormalities in metabolism include a shift toward anaerobic glycolysis with resultant increases in lactate and the development of intracellular acidosis. In addition, oxygen utilization is shifted toward relatively greater support for fatty acid oxidation, in preference to glucose oxidation. Ionic shifts include an increase in intracellular sodium and changes in the regulation of intracellular calcium cycling, which increase diastolic cytosolic levels and decrease the ability of sarcoplasmic reticulum to remove calcium from the troponin site in early diastole. The most important changes probably occur in mitochondria, which are responsible for producing the vast majority of ATP and which are sensitive to changes in intracellular calcium homeostasis.

Although reperfusion is necessary to halt ischemic necrosis, there is a sequence of events during the early reperfusion period that may result in myocardial injury. These events may be related to calcium overload, free radical generation, and inflammatory mediators. The potential for therapeutic interventions is demonstrated by the phenomenon of ischemic preconditioning. Exposure to a brief ischemic period before a sustained ischemic insult is associated with a remarkable resistance to the metabolic, functional, and histologic consequences of that prolonged ischemic period. The challenge is to develop therapeutic interventions capable of eliciting the protection afforded by the preconditioning stimulus.

REFERENCES


