Consequences of Brief Ischemia: Stunning, Preconditioning, and Their Clinical Implications
Part 2

Robert A. Kloner, MD, PhD; Robert B. Jennings, MD

Abstract—In experimental studies in the dog, total proximal coronary artery occlusions of up to 15 minutes result in reversible injury, meaning that the myocytes survive this insult. The 15 minutes of ischemia, however, induce numerous changes in the myocardium, including certain monuments to the brief episode of ischemia that may persist for days. One of these monuments is stunned myocardium, which represents “prolonged postischemic contractile dysfunction of myocardium salvaged by reperfusion.” The mechanism of stunning involves generation of oxygen radicals as well as alteration in calcium homeostasis and possibly alteration in contractile protein structure. Stunning has been observed in several clinical scenarios, including after percutaneous transluminal coronary angioplasty, unstable angina, stress-induced ischemia, after thrombolysis, and after cardiopulmonary bypass. Oxygen radical scavengers and calcium channel blockers have been shown to enhance function of stunned myocardium in experimental studies, and in a few clinical studies, calcium channel blockers have been shown to ameliorate stunning. Although brief periods of ischemia can contribute to prolonged left ventricular dysfunction and even heart failure, they paradoxically play a cardioprotective role. Episodes of ischemia as short as 5 minutes, followed by reperfusion, protect the heart from a subsequent longer coronary artery occlusion by markedly reducing the amount of necrosis that results from the test episode of ischemia. This phenomenon, called ischemic preconditioning, has been observed in virtually every species in which it has been studied and is a powerful cardioprotective effect. The mechanism of ischemic preconditioning involves both triggers and mediators and involves complex second messenger pathways that appear to involve such components as adenosine, adenosine receptors, the epsilon isoform of protein kinase C, the ATP-dependent potassium channels, as well as others, including a paradoxical protective role of oxygen radicals. Both an early and a late phase of preconditioning have been described, and the mechanisms underlying their induction are under investigation. That preconditioning may occur in humans is suggested by the observations that repetitive balloon inflations in the coronary artery are associated with progressively less chest pain, ST-segment elevation, lactate production, the protective effects of preinfarction angina, the anginal “warm-up phenomenon,” and studies performed on human cardiac biopsies that show metabolic properties suggesting preconditioning. Development of pharmacological agents that stimulate second messenger pathways thought to be involved in preconditioning, but without causing ischemia, could result in novel approaches to treating ischemia. Hence, on one hand, brief episodes of ischemia can have a negative effect on the heart: stunning; and on the other hand, they have a protective effect: preconditioning. The future challenge is how to minimize the stunning phenomenon and maximize the preconditioning phenomenon in clinical practice. (Circulation. 2001;104:3158-3167.)

Key Words: ischemia ■ myocardium ■ preconditioning ■ myocardial infarction ■ stunning, myocardial

After a brief episode of ischemia, oxygen-derived free radicals generated at the time of reperfusion damage the myocytes and thereby cause much of the stunning effect. Most of these studies investigated brief periods of ischemia (≈15 minutes) in animal models and showed that oxygen radical scavengers could improve the return of function of the stunned myocardium. Data regarding the effects of oxygen radical scavengers on more prolonged periods of ischemia, as would occur in a reperfused infarct, are mixed. There is little information on the effect of oxygen radical scavenging agents in human models of stunning. Although superoxide dismutase did not benefit patients with myocardial infarction undergoing reperfusion therapy, there are no data on the effect of oxygen radical scavenging agents that cross the cell membrane (such as N-2-mercaptopropionyl glycine) in this situation.

Another approach to treating stunned myocardium has been the administration of calcium channel blockers. In
experimental models of brief episodes of coronary artery occlusion and reperfusion, nifedipine, verapamil, diltiazem, and amiodipine were shown to enhance the return of cardiac function.\textsuperscript{13} The exact mechanism by which the calcium channel blockers work in these models is not clear. We observed that small intracoronary doses of nifedipine that did not alter hemodynamics or regional myocardial blood flow nevertheless improved the function of stunned myocardium; hence, it is probably not simply a function of afterload reduction or induction of hyperemia.\textsuperscript{13} One possibility is a direct cellular effect whereby the calcium blocker limits influx of calcium into the injured but viable cell.

Recent clinical studies suggest that calcium channel blockers also enhance the recovery of stunned myocardium in humans. Sheiban et al\textsuperscript{14} induced stunned myocardium in patients by inflating a coronary angioplasty balloon for 5 minutes, followed by reperfusion. Patients received either nitrate therapy or therapy with the calcium channel blocker nisoldipine. Most patients pretreated with nitrates demonstrated stunning at 24 hours after the angioplasty, whereas only 8% pretreated with nisoldipine showed stunning at 15 minutes after balloon deflation, and all had recovery of left ventricular function by 1 day after reperfusion. Rinaldi et al\textsuperscript{15} also studied the effect of calcium channel blockade in patients undergoing exercise stress echocardiograms by comparing the effect of isosorbide mononitrate or amiodipine on postexercise wall motion abnormalities. As expected, shortening fraction was better preserved in patients on amiodipine than in those on nitrates. The use of calcium channel blockers to treat stunning appears promising but remains investigational at this time.

Stunned myocardium responds to inotropes, and although this approach may not be a true treatment for the condition, it is commonly used in the post–cardiopulmonary bypass setting or in patients who demonstrate persistent left ventricular dysfunction and severe heart failure after successful and timely reperfusion.\textsuperscript{16–18} Arnold et al found that inotropic stimulation of stunned myocardium did not worsen ultimate recovery of the tissue or induce necrosis, as long as the artery was fully patent.\textsuperscript{17} The theory on why inotropic stimulation works relates to the concept that a mechanism of stunning is desensitization of the myofilaments to calcium. It is likely that inotropes simply overcome this desensitization by increasing the availability of calcium to the myofilaments.

What are the clinical implications of stunned myocardium? Physicians should be aware that brief periods of ischemia can be associated with prolonged contractile dysfunction. From a practical standpoint, this is rarely an issue in the catheterization laboratory, because angioplasties usually are brief enough not to cause prolonged systolic dysfunction. As shown by Wijns et al,\textsuperscript{19} however, even brief balloon inflation may cause relatively persistent diastolic dysfunction. Clinicians should be aware that the ultimate recovery of myocardium salvaged by reperfusion may not occur in the first few days of therapy. Three days to several weeks may be necessary, and some patients may demonstrate heart failure due to stunning. Hence, inotropic support during the early phase of reperfusion may be warranted. Stunning after cardiac surgery is common and may precipitate heart failure; again, inotropes are commonly used in these circumstances. Stunning may occur in the setting of angina, even exercise-induced angina. In most cases, the regional wall motion abnormalities induced after a bout of exercise-induced ischemia resolve spontaneously and do not cause undue morbidity. They could precipitate heart failure in some patients, however. Patients with unstable angina have exhibited stunning, and again, the concern here is that this could induce heart failure in some patients. The development of heart failure symptoms in patients with extensive coronary artery disease but without infarction is often referred to as ischemic cardiomyopathy. One potential contributing factor to this type of cardiomyopathy may be stunning.

Finally, in the clinical realm, the term “stunning” has been used (whether properly or not) to describe a phenomenon not included in the original description. Left atrial stunning after cardioversion refers to transient contractile dysfunction of the left atrium after electrical or chemical cardioversion of atrial fibrillation or flutter to sinus rhythm.\textsuperscript{20} The slow return of left atrial function may be due in part to a tachycardia-induced cardiomyopathy that occurred during the arrhythmia and/or contributions from damage done during the cardioversion itself. Reports suggest that recovery of left atrial function may take up to several weeks and be associated with spontaneous echocardiographic contrast, which implies stasis of flow in the left atrium and may be a forerunner of thrombus formation. Hence, the clinical implication of this form of stunning is that anticoagulation may need to be administered for 3 to 4 weeks after cardioversion.\textsuperscript{21}

### Relationship of Stunning to Hibernation

Hibernation is a concept coined by Diamond\textsuperscript{22} and popularized by Rahimtoola\textsuperscript{23} to explain how myocardium supplied by a vessel with a fixed stenosis can remain alive and persistently acontractile while receiving deficient arterial flow. Rahimtoola’s definition of this condition follows: Hibernation is “a state of persistently impaired myocardial and left ventricular (LV) function at rest due to reduced coronary blood flow that can be partially or completely restored to normal if the myocardial oxygen supply/demand relationship is favorably altered, either by improving blood flow and/or by reducing demand.”\textsuperscript{23} According to the results of several kinds of studies, especially PET studies with fluorodeoxyglucose, the acontractile tissue is still alive even though arterial flow is depressed.\textsuperscript{24,25} Moreover, it can contract if arterial flow is restored.

The hibernation hypothesis proposes that the tissue has downregulated its metabolism in response to the reduced arterial flow and that this downregulation, just like hibernation in a bear or woodchuck, allowed the myocyte to survive in a situation in which flow was insufficient to maintain contraction. This concept has been difficult to study because of the absence of a good animal model of chronic hibernation. It is very difficult to detect complex alterations in metabolism in human myocardium because direct sampling of the tissue for long-term metabolic studies cannot be done. Nevertheless, salvageable and potentially functional acontractile viable tissue clearly exists in patients with severe coronary disease. It remains to be proven that distinctive metabolic alterations
are occurring in this tissue, but the fact that it is salvageable and will function if appropriately treated is a very important observation.

Stunning, like the concept of hibernating, refers to viable myocardium that is not contracting properly but is not acutely ischemic. Identifying such viable tissue in a poorly functioning heart is important in that this tissue will eventually recover function if blood flow is restored. Remember, however, that necrotic or scar tissue, which is often present in areas resupplied by a vessel with a significant stenosis, cannot recover contractile function.

It was recently proposed that the functional state of defined contractile failure of hibernation is due to chronic stunning caused by multiple episodes of more severe ischemia brought on by increases in oxygen demand, such as exercise, or reduction of flow, as in coronary spasm, each followed by the equivalent of reperfusion. This would result in a persistent stunned, acontractile state. Moreover, it has been possible to model this condition in larger-animal hearts, such as pig hearts. Other experimental studies, however, suggest that hibernation does occur with a chronic low-flow state. Whether the acontractile state is due to downregulation of metabolism or to chronic stunning, the treatment is clear: arterial flow must be improved.

**Time Course of Stunning**

The time course of stunning is acute or subacute, on the order of hours to weeks, depending on the degree and duration of the initial ischemic insult. Brief episodes of ischemia of ≤15 minutes that might mimic angina and are not associated with cell death require ~48 hours for recovery (Figure). Once the duration of ischemia is extended beyond 20 minutes and cell death begins to occur in the subendocardium, recovery of function may require days to perhaps even longer. Again, stunning is typified by a prolonged recovery of function after relief of a discrete episode of ischemia. Wall motion abnormalities that persist for months but occur within viable myocardium may represent so-called hibernating myocardium, which represents a chronic phenomenon. There are some histological features of hibernating tissue that differentiate it from myocardium that has experienced one or few episodes of stunning. Whereas the electron microscopic appearance of stunned myocardium shows minimal change, hibernating myocardium shows a pattern reminiscent of degenerating cardiomyocytes, with large perinuclear pools of glycogen and mitochondria and the presence of myofilaments limited to the cell periphery. This loss of myofilaments probably contributes to the hibernating phenomenon and may help explain why it is more of a chronic abnormality. The loss of myofilaments also helps explain why some studies show that revascularization of hibernating myocardium does not always result in early dramatic improvement in function but that months may be required for return of contractility.

**Ischemic Preconditioning**

**Definition and Biology of Ischemic Preconditioning**

Although episodes of transient myocardial ischemia can induce the reversible injury of stunned myocardium, they can also protect the heart from extensive necrosis. Murry et al first described the concept of ischemic preconditioning. In a study reported in 1986, they reported that anesthetized dogs subjected to 40 minutes of circumflex coronary artery occlusion and reperfusion demonstrated a marked reduction of myocardial infarct size when the dogs received 4 brief episodes of 5 minutes of ischemia separated by 5 minutes of reperfusion just before the 40-minute occlusion. It was this reduction in infarct size caused by the previous exposure of the heart to brief episodes of ischemia that was referred to as ischemic preconditioning. Analysis of the extent of necrosis within the risk zone as a function of coronary collateral flow showed an inverse relationship within the control animals. The lower the coronary collateral flow, the greater the percentage of the risk zone that went on to develop necrosis. In the animals receiving ischemic preconditioning, this relationship was altered. Even in dogs with low coronary collateral flow, the extent of necrosis was reduced markedly with preconditioning. Ischemic preconditioning subsequently was shown to occur in rat, rabbit, pig, and mouse hearts. The phenomenon was reproduced easily by a multitude of experimental laboratories. In the history of attempts to limit myocardial infarct size via a host of interventions, such consistency among laboratories was unprecedented.

Preconditioning can be induced by periods of ischemia as short as 3 to 5 minutes followed by 5 minutes of reperfusion. It is clear that a single episode of transient ischemia is all that
is needed to induce preconditioning, although laboratories often use repetitive episodes of brief ischemia to induce the phenomenon. If the duration between brief ischemia to induce preconditioning and the more prolonged ischemic episode to induce necrosis is extended from 5 minutes to 3 hours in the dog heart, the benefit of ischemic preconditioning disappears. If the duration between the ischemic preconditioning episodes and the long-duration coronary artery occlusion is extended to 24 to 96 hours, however, then the protective effect returns and infarct size is reduced, although not to as great an extent as when the long occlusion occurs shortly after ischemic preconditioning. This later phase of ischemic preconditioning originally was called the second window of protection but now is best called delayed or late preconditioning, whereas acute preconditioning is often referred to as classic or early preconditioning. The biology of classic preconditioning is summarized as follows: It delays but does not prevent myocyte death during the test episode of ischemia. Thus, if the duration of the test episode of ischemia is excessive or reperfusion is not eventually instituted, preconditioning will not work. It occurs in all mammalian hearts tested thus far. The effects are maximal in large-animal hearts, in which the metabolism and heart rates are lower. Finally, as discussed under mechanisms, the beneficial effect is present while energy demand is diminished in the preconditioned tissue. Myocardium may be chronically preconditioned by repetitive brief occlusions, but if the occlusions are too frequent and too close together, tachyphylaxis can occur.

Although the definition of ischemic preconditioning initially referred to reduction of infarct size, some investigators have extended it to describe the protective effects of brief ischemia on cardiac function and arrhythmias. These latter protective effects have not been as consistent as effects on infarct size. There are also ECG correlates of ischemic preconditioning, including less ST-segment elevation on subsequent brief coronary occlusions compared with a first occlusion. Importantly, this phenomenon can be seen in models of extremely low coronary collateral blood flow, such as the rabbit, and therefore is not due to recruitment of coronary collateral flow. Sebbag et al suggested that attenuation of ST-segment elevation was immediate evidence for the protective effect of preconditioning. Furthermore, when the beneficial effect of preconditioning was lost after 120 or 180 minutes of reperfusion, the attenuation of ST-segment elevation was also lost.

Mechanism(s) of Preconditioning
The mechanism(s) of ischemic preconditioning appears to be complex and to involve second messenger pathways, as suggested by the pioneering work of Downey’s group. Also, the mechanism of the delayed phase of preconditioning differs in many ways from that of the early phase or classic preconditioning.

Our analysis of mechanisms will be limited to a consideration of protection against the key end point: cell death, and not other end points such as arrhythmias, autonomic denervation, or vascular responses. Mechanistic studies have been performed in numerous models of preconditioning, including (1) preconditioned isolated rabbit, rat, neonatal chicken, and human myocytes; (2) perfused rat, rabbit, or mouse hearts preconditioned in vitro; and (3) infarct sizing in vivo in small rodents. Where indicated, results from these models will be included, but our discussion will concentrate on findings in large-animal hearts, because these changes are more likely to be relevant to preconditioning in the human heart.

Myocardium that is fully preconditioned exhibits the striking metabolic changes noted earlier in the section on the metabolic and physiological changes of reversible injury, including a smaller adenine nucleotide pool (ΣAd), excess intracellular glucose, a creatine phosphate overshoot, and stunning. In addition, it reacts to a second episode of ischemia much differently than virgin myocardium in that it utilizes ATP and accumulates lactate and H⁺ much more slowly. This situation of slowed anaerobic glycolysis (the principal pathway of ATP synthesis in severe ischemia) despite slowed depletion of high energy phosphate (HEP) is best explained by hypothesizing that energy demand is reduced in ischemic preconditioned tissue. Because low intracellular ATP and high tissue lactate and H⁺ are strongly associated with ischemic cell death, it has been postulated that preconditioned tissue dies more slowly because of this reduction in energy demand.

The change or changes that occur during the preconditioning episode of ischemia that trigger the preconditioning response have not been identified with certainty. Also, the changes that persist within the preconditioned tissue during the reperfusion phase and either provide a memory of the preconditioning event that changes the responses of the myocardium to test ischemia or mediate the preconditioning effect during the test episode of ischemia have not been established.

Triggers of Preconditioning With Ischemia
A trigger is considered to be a substance released during ischemia and possibly during reperfusion that stimulates signaling pathways in the myocytes that cause the changes that allow myocytes to survive a test episode of ischemia longer than virgin myocytes. Evidence for a putative trigger would include the following: (1) the putative trigger increases in concentration during ischemia; (2) administration of a putative trigger to a coronary bed or myocardium itself without ischemia will induce a state of pharmacological preconditioning, ie, a state of protection similar to ischemic preconditioning but caused by a drug rather than ischemia; (3) administration of an inhibitor of the trigger will eliminate the protection; and (4) this inhibitor will prevent preconditioning with ischemia. When an inhibitor to a putative trigger prevents preconditioning caused by an episode of ischemia and reperfusion, it follows that this trigger may be the cause of preconditioning with ischemia in vivo.

Mediators of Preconditioning With Ischemia
The protective effect caused by ischemia is hypothesized to be caused by a mediator, ie, a change occurring intracellularly as a consequence of the action of a trigger that then somehow
protects against ischemia. There are 2 chief candidate mediators: the Kₐ₅p channel⁶¹–⁶₅ and specific isoforms of protein kinase C (PKC).⁵⁹

The Kₐ₅p channel, a channel found in high concentration in the sarcolemma, opens whenever intracellular ATP declines substantially, e.g., to the levels found during a 5-minute episode of ischemia in the dog heart. This effect of ischemia can be blocked by pretreatment of the myocardium before the preconditioning episode of ischemia with inhibitors of the Kₐ₅p channel, such as glibenclamide and 5-hydroxydecanolate (5HD).⁶³,⁶⁴ These data support the idea that the Kₐ₅p channel is the mediator of the preconditioning effect. In addition, there is a Kₐ₅p channel in the mitochondria.⁶⁵ This channel is opened quite specifically by diazoxide and is blocked with low concentrations of 5HD compared with the quantities of 5HD required to block the sarcolemmal Kₐ₅p channel.⁶⁶ Our laboratory (R.B.J.) has shown that pretreatment with diazoxide pharmacologically preconditions the dog heart and reduces infarct size to much the same extent as preconditioning with ischemia, although this effect is less marked in the in vivo rabbit model.⁶⁷ Thus, there are strong data supporting the idea that a trigger exerts its effect by opening the mitochondrial Kₐ₅p channel and that this somehow mediates the protective effect.⁶²,⁶⁶

Although there is much evidence that Kₐ₅p channels are mediators, Downey’s group (Downey and Cohen; Pain et al⁶⁸) recently presented evidence gathered in the isolated perfused rabbit heart that indicates that Kₐ₅p channel activation in the mitochondria may be a trigger rather than a mediator. This issue remains to be resolved.⁶¹,⁶⁹ as does the question of how opening the mitochondrial Kₐ₅p channel provides protection.

The second proposed mediator is the epsilon isoform of PKC. This isoform of this kinase (but not total PKC) is translocated during ischemia.⁶⁰,⁷⁰–⁷³ When active, it phosphorylates serine and threonine groups in enzyme or channel proteins and presumably exerts its effect through this phosphorylation. This phenomenon has been difficult to study in vivo because many of the potential agonists and inhibitors are insoluble in water and cannot be administered easily in vivo. Nevertheless, Vahlhaus et al⁷⁴ have shown that inhibition of both PKC and tyrosine kinase will prevent ischemic preconditioning in the pig heart, whereas inhibition of PKC alone has no effect.⁷⁵

**Adenosine**

This nucleoside is released quickly into the extracellular fluid very early in ischemia and is present in concentrations greater than those required to stimulate the receptor. Thus, it is a potential trigger. Also, it has been shown in vivo in both rabbit⁷⁶ and dog⁷⁷ hearts that adenosine or A₁ agonists of adenosine will pharmacologically precondition the heart against the effects of a test episode of ischemia.⁷⁸ Moreover, these effects can be blocked by administration of an inhibitor of the adenosine effect such as 8-(p-sulfophenyl)theophylline⁷⁹ or by the Kₐ₅p channel inhibitor glibenclamide.⁷⁷ The results of these experiments, taken together, provide strong evidence that adenosine is involved in preconditioning with ischemia and the potassium channel is involved in mediating the effect of adenosine. Also, good data exist that the beneficial effect of adenosine is mediated via the A₁ receptor⁷⁶,⁷⁸ and that this receptor stimulates PKC isoform translocation (activation).⁴³

**Other Triggers**

Administration of bradykinin⁷⁹,⁸₀ and opioids⁸¹,⁸² will induce pharmacological preconditioning. Bradykinin and opioids are released during the preconditioning episode of ischemia on a time scale consistent with these agents being involved in the phenomenon. In addition, the observation that the beneficial effects of preconditioning with ischemia are eliminated or reduced by inhibitors of bradykinin and opioids indicates that these agents can act as triggers.⁸¹ Thus, it seems likely that there are a series of triggers in preconditioning. Downey’s group has provided some evidence indicating that they may act synergistically.⁸⁰

**How Is Myocyte Death Delayed in Preconditioning?**

A major mechanism proposed to explain why preconditioned myocytes tolerate ischemia better than virgin myocytes is that the reduced energy demand found in ischemic preconditioned tissue preserves ATP and slows the development of the osmotic load and acidosis.⁷⁷ Slowing of the development of these changes, both of which are an invariable accompaniment of ischemic cell death, is consistent with a delay of the transition to irreversibility.⁸₅

Another unexpected change involves O₂-derived free radicals appearing during reperfusion. Murry et al⁸⁴ showed in 1988 that intravenous administration of the free radical scavengers superoxide dismutase and catalase would prevent preconditioning with ischemia in many but not all dog hearts. These data suggested that free radicals exerted a paradoxical protective rather than deleterious effect in reperfused ischemic myocardium. It was recently suggested that the effect of opening potassium channels in mitochondria with diazoxide and thereby preconditioning the heart is to increase O₂-derived free radical production from the mitochondria.⁶₈ These O₂-derived free radicals could mediate the protective effect through some as yet unknown mechanism that appears to involve mitochondria.⁶₈ An attempt to show that the mitochondrial ATPase, one of the chief sources of ATP utilization during ischemia, was inhibited more quickly during the prolonged episode of ischemia, a change that would slow ATP depletion, was unsuccessful.⁸₅

**Genetic Responses**

It has seemed unlikely that classic preconditioning is mediated by any new protein synthesis. This is because precondi-
Preconditioning can be induced so quickly, eg, by 3 to 5 minutes of ischemia and 5 minutes of reperfusion. Also, inhibition of protein synthesis with cyclohexamide and RNA synthesis with actinomycin D has no effect on preconditioning with ischemia, an observation that indicates that effective gene activation has not occurred in the brief interval required to precondition in classic preconditioning.

Delayed or Late Preconditioning

This phenomenon is of great interest (see section on delayed preconditioning and stunning) for 3 reasons. The first is that the preconditioning episode of ischemia clearly causes new enzyme synthesis. Included are inducible NO synthase, superoxide dismutase, and heat-shock proteins. Some or all of these proteins may be involved in the protection. The second is the fact that the protective effect persists for \( \approx 48 \) hours. It appears to be slightly weaker than the protection wrought by classic preconditioning but is easy to demonstrate in rabbits, pigs, and dogs. The third reason that delayed preconditioning is noteworthy is that \( O_2^- \)-derived free radicals, probably peroxynitrite, are involved in inducing the protection, in that one can eliminate late preconditioning by pretreatment with a free radical scavenger before the preconditioning episodes of ischemia. Because there is indirect evidence that \( O_2^- \)-derived free radicals are involved in the protection noted in classic preconditioning, it is likely that different free radicals mediate the effect, because NO does not appear to be involved in classic preconditioning.

Preconditioning in Patients

Preconditioning has been demonstrated in experimental studies of all species that have been tested. Clinical studies also support the concept that preconditioning occurs in humans (Table). When isolated human myocardial cells in culture are exposed to severe hypoxia for 90 minutes followed by reoxygenation, some cells will die, and the extent of cell death can be quantified by trypan blue exclusion. Ikonomidou et al showed that exposing the cells to brief periods of hypoxia and reoxygenation before the longer 90-minute duration of test hypoxia significantly reduced cell death. This observation is very important, for 2 reasons: first, it suggests that human myocardial cells can be preconditioned; second, it suggests that other cell types are not needed for preconditioning. The benefit of ischemic preconditioning thus depends on primary biochemical changes within the myocyte itself and not on changes in the vasculature or other interstitial cells. This finding agrees with the general observation that ischemic preconditioning is not dependent on recruitment of collateral blood vessels, because species without collaterals, such as rats, pigs, and rabbits, all can be preconditioned.

Other studies suggest that human myocardial tissue can be preconditioned. Yellon and associates showed that biopsies taken from human hearts undergoing surgery display properties suggesting preconditioning. Brief episodes of ischemia preserved cardiac enzymes and function of isolated human cardiac muscle when exposed to longer periods of in vitro–simulated ischemia. Preconditioning-mimetic agents (pharmacological agents thought to work along the same pathways as ischemic preconditioning) also appeared to precondition human tissue. Yellon and associates showed that intermittent aortic cross-clamping could precondition human left ventricles during coronary artery bypass surgery, resulting in a preservation of ATP levels.

In addition to studies involving assessment of human cardiac tissue, there are several clinical scenarios that suggest that ischemic preconditioning occurs in humans. PTCA has provided insight into the effects of brief episodes of ischemia on the human heart. When repetitive balloon inflations (usually on the order of 60 to 90 seconds) and deflations are performed, the following clinical observations have been made: (1) the severity of chest pain lessens with subsequent compared with a first balloon inflation, (2) the degree of ST-segment elevation is reduced with subsequent balloon inflations, (3) the degree of lactate production is reduced with subsequent compared with a first inflation, and (4) the phenomenon occurs in patients who do not appear to recruit collaterals.

The sequential angioplasty balloon inflation model may be a useful screening tool whereby pharmacological agents that mimic preconditioning but do not cause ischemia can be tested. It has been observed that preconditioning mimetics, such as adenosine or nicorandil, reduce the subsequent

<table>
<thead>
<tr>
<th>Clinical Situations in Which Preconditioning May Occur</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repetitive balloon inflations during PTCA</td>
<td>Less chest pain, ST elevation, lactate with sequential inflations. Pharmacological preconditioning may mimic effects of brief ischemia.</td>
</tr>
<tr>
<td>Preinfarct angina</td>
<td>Associated with smaller infarcts, improved clinical outcomes. Debate in elderly patients</td>
</tr>
<tr>
<td>Warm-up phenomenon</td>
<td>Less angina and ECG signs of ischemia when second exercise period occurs after short rest</td>
</tr>
<tr>
<td>Studies in human tissue</td>
<td>Exhibit preconditioning-like properties when exposed to simulated ischemia/reoxygenation. Pharmacological preconditioning has been demonstrated.</td>
</tr>
<tr>
<td>Isolated human cardiomyocytes</td>
<td>Exhibit preconditioning-like properties and enhanced function with preconditioning ischemia before longer-duration ischemia.</td>
</tr>
<tr>
<td>Isolated human muscle strips</td>
<td>Intermittent aortic cross-clamping mimics preconditioning, preserves cardiac ATP levels.</td>
</tr>
<tr>
<td>Biopsies taken from human cardiac tissue at time of coronary artery bypass surgery</td>
<td>Intermittent aortic cross-clamping mimics preconditioning, preserves cardiac ATP levels.</td>
</tr>
</tbody>
</table>

Kloner et al Consequences of Brief Ischemia 3163
amount of ST changes. Conversely, agents that are known to block preconditioning pathways, such as the K\textsubscript{ATP} channel blocker glibenclamide, were shown to prevent the beneficial effects of repetitive balloon inflation.\textsuperscript{98} From a practical standpoint, difficult angioplasties associated with severe ischemia during initial balloon inflation and not amenable to stent placement might benefit from a preconditioning protocol of sequential brief inflations and deflations.

Several clinical studies now suggest that brief episodes of ischemia in the form of angina occurring within the first day or so before acute myocardial infarction may have protective effects.\textsuperscript{99–102} In one thrombolytic study from the TIMI-4 group,\textsuperscript{99} preinfarction angina was associated with a smaller infarct size as assessed by creatine kinase curves, fewer in-hospital deaths, and less congestive heart failure and/or shock. There was no difference in coronary collateral scores between patients with versus those without preinfarction angina, and the benefit was not due to use of antianginal drugs or aspirin. In a more recent study from the TIMI-9 group in which the timing of angina in relationship to myocardial infarction was evaluated, only those patients with angina within 24 hours of infarction derived benefits on infarct size or clinical outcomes from the preinfarction angina.\textsuperscript{103} Numerous other thrombolytic trials have suggested that preinfarction angina can reduce myocardial infarct size in patients, improve survival, improve left ventricular function, and reduce arrhythmias.\textsuperscript{94,99–103} Although classic preconditioning might play some role in these phenomena, it is likely that episodes of pain occurring closer to 1 day before infarction, as well as silent ischemia which may occur at this time, may be having a benefit through the delayed preconditioning mechanism. Andreotti et al\textsuperscript{104} made the fascinating observation that the benefit of preinfarction angina may be related to better and faster thrombolysis, implying that preinfarction angina might induce a vascular preconditioning effect. For example, brief periods of ischemia due to angina that occur before myocardial infarction might release adenosine that could then interfere with platelet aggregation.

Not all preinfarction angina studies have been positive. In one recent report, preinfarction angina had no benefit when the infarct was reperfused by PTCA rather than thrombolysis.\textsuperscript{105} This observation would tend to support the findings of Andreotti et al.\textsuperscript{104} There is also debate as to whether preinfarction angina has any benefit in elderly patients. Some studies have shown benefits in the elderly, but others have not.\textsuperscript{106–109}

Another clinical manifestation of ischemic preconditioning is the so-called warm-up phenomenon.\textsuperscript{89,94,110–112} In this common clinical scenario, patients with angina exercise to the point of angina, stop, rest, and then are able to continue exertion without further angina. When patients received exercise tests, rested for 15 minutes, and then repeated the exercise tests, they showed better exercise tolerance and had less ST-segment depression on the second than the first test.\textsuperscript{110} Regional myocardial oxygen consumption was lower during the second test than the first, and flow through the cardiac vein did not differ between tests. Williams et al\textsuperscript{111} induced angina by pacing-induced tachycardia at the time of catheterization. Two identical periods of pacing were sepa-

Preconditioning as Therapy

Can ischemic preconditioning be used to treat cardiovascular disorders? Over the past 15 years, a large volume of research has focused on the mechanisms of both early and late ischemic preconditioning.\textsuperscript{43,44} Results of these studies have suggested that ischemic preconditioning involves second messenger pathways\textsuperscript{43} that theoretically could be stimulation and thereby induce the beneficial effects of ischemic preconditioning without ischemia. Conversely, the inhibition of various aspects of these pathways can prevent ischemic preconditioning. Development of preconditioning mimetic agents has shown promise in experimental studies. Examples include adenosine, adenosine agonists, PKC agonists, K\textsubscript{ATP} channel openers, and NO donors.\textsuperscript{43,44} Translating these beneficial agents to the clinical realm has been tricky, because many of these agents have profound hemodynamic effects. For example, whereas diazoxide, a mitochondrial K\textsubscript{ATP} channel opener, mimicked preconditioning and reduced cell death in several animal models, it also causes significant hypotension in humans. Nevertheless, some preconditioning mimetic agents already are used clinically, although their use may not be based primarily on the preconditioning concept. Nicorandil is a K\textsubscript{ATP} channel opener that reduced angina.\textsuperscript{113} It is currently on the market in Japan and in parts of Europe. Adenosine has shown promise when given as an adjunct to cardioplegia; in this setting, it appears to decrease the need for use of high-dose inotropes after cardiac surgery.\textsuperscript{114} Adenosine also is being tested as an adjunct to reperfusion in patients with acute myocardial infarction. In the AMISTAD I trial,\textsuperscript{115} adenosine reduced infarct size in patients with anterior myocardial infarction. Whether the benefit of adenosine was related to a preconditioning mechanism is not clear, because the drug was given not at preconditioning but at reperfusion.

In what clinical scenarios could preconditioning mimetics be useful? Most data in the experimental literature suggest that preconditioning mimetics would need to be given before occlusion to attain a true preconditioning effect with reduction of infarct size at the time of thrombolysis. Unfortunately, this will not be possible in the clinical setting of acute myocardial infarction, unless the patient is taking an oral medicine long-term. And even that might not work, because long-term therapy with a preconditioning mimetic might lead to tachyphylaxis. However, this has never been established experimentally. If tachyphylaxis does not develop, giving preconditioning mimetics to patients at risk of infarction for the purpose of delaying cell death before reperfusion is possible, but only if the agent is relatively nontoxic and has
few side effects. Preconditioning mimetic agents clearly can be applied to reduce ischemic damage, when the period of ischemia is controlled and predicted. Two clinical situations that might benefit from preconditioning mimetics would be before cardiopulmonary bypass or to a heart at the time of removal for transplantation. Providing these agents to patients with unstable angina in which the mimetic might augment the protective effect of angina itself is another area that deserves further study. As mentioned, K\textsubscript{ATP} channel blockers may show promise in this regard. A preconditioning agent might prevent or reduce the amount of necrosis should the patient develop a myocardial infarction. Such an agent could reduce the amount of ischemia that develops during a high-risk angioplasty procedure in which the occluded vessel supplies a very large risk area. Of course, another approach would be to perform multiple brief balloon inflations that would ischemically precondition the heart. With the advent of stents and perfusion catheters, however, it is unlikely that preconditioning mimetics would have a great practical clinical role in the catheterization laboratory. Finally, preconditioning mimetics might be useful for patients with exercise-induced angina that is not fully controlled by the usual drugs. Taking such agents before an episode of planned exertion might stave off angina. Hence, although preconditioning is one of the most powerful techniques for reducing ischemic necrosis during coronary artery occlusion, its translation into practical use in the clinic will still require significant research and clinical trials before preconditioning mimetics become standard therapy for ischemic syndromes.

References


60. Fryer RM, Hsu AK, Gross GJ. Mitochondrial KATP channel opening is important during index ischemia and following myocardial reperfusion in ischemic preconditioned rat hearts. J Mol Cell Cardiol. 2001;33:831–834.


Kloner et al. Consequences of Brief Ischemia 3167

Consequences of Brief Ischemia: Stunning, Preconditioning, and Their Clinical Implications: Part 2
Robert A. Kloner and Robert B. Jennings

Circulation. 2001;104:3158-3167
doi: 10.1161/hc5001.100039

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/104/25/3158

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/