On July 2–3, 1996, the National Heart, Lung, and Blood Institute sponsored a workshop in Columbia, Md, entitled “The Medical and Cellular Implications of Myocardial Stunning, Hibernation, and Preconditioning.” The goals of this workshop were to identify and discuss the areas of agreement and controversy regarding these important phenomena and in particular to identify areas of future research for each. One aspect of these goals included determination of the mechanisms of these phenomena. Stunning is a form of prolonged contractile dysfunction that occurs after relief of a discrete episode or episodes of ischemia; hibernation is a form of prolonged contractile dysfunction associated with ongoing low blood flow, although controversy exists as to whether absolute blood flow or coronary reserve is reduced and whether it may represent repetitive bouts of stunning. Preconditioning is a cardioprotective mechanism in which the heart is exposed to a controlled, short period of sublethal ischemia that attenuates cellular damage from a subsequent prolonged lethal episode of ischemia. Research efforts have not yet provided a clear understanding of all aspects of these conditions. The workshop presented the current state of both basic science knowledge and clinical knowledge of these disorders, promoted discussions between basic and clinical scientists, and identified likely mechanisms and new directions for research.

The meeting was chaired by Eugene Braunwald and cochaired by Roberto Bolli, Eduardo Marban, and Robert A. Kloner and was coordinated by Leslie Reinlib. Twenty participants represented a broad spectrum of expertise: basic and clinical scientists, pathologists, and surgeons. (A list of conference participants is provided in the Appendix.)

The purpose of this article is to review some of the points made at the workshop in regard to areas of general agreement and controversy and, most importantly, to summarize the areas that need further research. The following discussions briefly review the concepts of stunning, hibernating, and preconditioning discussed at the workshop and some additional material that has become available since the workshop.

Following each brief discussion are suggestions made by the panel for future research directions.

Myocardial Stunning

The Protean Nature of Myocardial Stunning

Myocardial stunning is a general term that describes the mechanical dysfunction that persists after reperfusion despite the absence of irreversible damage and despite return of normal or near-normal perfusion,1,2 This phenomenon was first described by Heyndrickx et al3 in 1975 in conscious dogs undergoing brief coronary occlusions. One of the major problems in formulating a unifying pathophysiological and pathogenetic paradigm for myocardial stunning is that this phenomenon occurs in a wide variety of settings that differ from one another in several major respects.2 Indeed, myocardial stunning might be regarded not as a single entity but instead as a phenomenon. At the experimental level, the available observations can be grouped into six categories2: (1) stunning after a single, completely reversible episode of regional ischemia in vivo (eg, a coronary occlusion <20 minutes in the dog), as originally described by Heyndrickx et al3–6; (2) stunning after multiple, completely reversible episodes of regional ischemia in vivo (eg, repeated 5- or 10-minute coronary occlusions in the dog)7–11; (3) stunning after a partly reversible (no necrosis) plus partly irreversible (some areas of necrosis) episode of regional ischemia in vivo (eg, a coronary occlusion >20 minutes but <3 hours in the dog)12,13 (this category may be relevant to patients who receive thrombolysis or angioplasty for acute myocardial infarction and then demonstrate delayed recovery of function of the salvaged tissue); (4) stunning after global ischemia in vitro (isolated heart preparations)14–19; (5) stunning after global ischemia in vivo (cardioplegic arrest)20–23; and (6) stunning after exercise-induced ischemia (high-flow ischemia).24–26 Because of the heterogeneity of these settings, it is possible that findings developed in one setting may not be applicable to another.2 Thus, an important, unresolved issue is whether all forms of stunning share a common pathogenesis.
Pathogenesis of Myocardial Stunning

Myocardial stunning is probably a multifactorial process that involves complex sequences of cellular perturbations and the interaction of multiple pathogenetic mechanisms. The most plausible hypotheses regarding the pathogenesis of myocardial stunning are the oxyradical hypothesis and the calcium hypothesis. The oxyradical hypothesis postulates that stunning is caused by oxidant stress secondary to the generation of ROS. In a very broad sense, the calcium hypothesis postulates that stunning is the result of a disturbance of cellular calcium homeostasis. As pointed out previously, these theories are not mutually exclusive and probably represent different facets of the same pathophysiological process. A unifying hypothesis for the pathogenesis of myocardial stunning is proposed in Fig 1A.

Generation of ROS (Oxyradical Hypothesis)

It is now generally accepted that ROS play an important role in the pathogenesis of myocardial stunning in the following experimental settings (as reviewed in Reference 27): (1) after a single, completely reversible episode of regional ischemia in vivo; (2) after multiple, completely reversible episodes of regional ischemia in vivo; (3) after global ischemia in vitro; and (4) after global ischemia in vivo. For example, in the first setting (coronary occlusion <20 minutes), alleviation of stunning by antioxidant therapies has been reproducibly observed by several independent laboratories in a variety of animal models and species. Similar protective effects of antioxidants have subsequently been demonstrated in conscious animals. At the time of this writing, at least 22 full-length articles have been published that have examined the effect of antioxidants on myocardial stunning after a 15-minute coronary occlusion; all of these articles (except those that used superoxide dismutase alone or catalase alone) have reported a protective effect of antioxidants against stunning. Furthermore, generation of ROS in stunned myocardium has been demonstrated directly by both spin trapping and aromatic hydroxylation techniques, and attenuation of ROS generation has repeatedly been shown to result in attenuation of contractile dysfunction. A role of ROS in the genesis of myocardial stunning can now be regarded as a proven hypothesis. Nevertheless, it remains unclear whether ROS play a role in all settings of stunning. There is presently no evidence that they contribute to exercise-induced postischemic dysfunction. Data regarding the effects of antioxidants on myocardial stunning after a prolonged (>20 but <180 minutes) ischemic insult, causing some subendocardial infarction and adjacent areas of stunning, are conflicting (reviewed in Reference 37); thus, the role of ROS in this setting is uncertain.

The available evidence indicates that most or all of the ROS-mediated injury responsible for stunning occurs in the initial moments of reperfusion. For example, in the setting of a 15-minute coronary occlusion in the dog, antioxidant therapies are equally effective in alleviating myocardial stunning irrespective of whether they are begun before ischemia or just prior to reperfusion; however, antioxidant therapies begun 1 minute after reperfusion are ineffective, indicating that the ROS important in causing myocardial stunning are those produced immediately after reflow.

Thus, myocardial stunning can be viewed in part as a form of ROS-mediated “reperfusion injury.” This concept may have significant therapeutic implications because it suggests that antioxidant therapies begun after the onset of ischemia could still be effective in preventing postischemic dysfunction; however, a delay in the implementation of such therapies until after reperfusion would result in loss of efficacy. It is important to stress, however, that none of the antioxidant therapies used thus far has completely prevented myocardial stunning. On the basis of these observations, it seems reasonable to conclude that the injury responsible for myocardial stunning consists of two components: (1) a component that develops during ischemia (ischemic injury) and is not responsive to antioxidant therapy (no matter how vigorous or how early applied) and (2) a component that develops after reperfusion (reperfusion injury) and can be mitigated by early antioxidant therapy (Fig 1B). Judging from the effects of antioxidants, the reperfusion injury component appears to be larger than the ischemic injury component.

The exact source of ROS production in stunned myocardium remains uncertain (Fig 1A), but two points are clear: (1) neutrophils do not contribute to stunning after reversible ischemia in vivo; and (2) xanthine oxidase is not necessary for ROS to be generated in sufficient quantities to induce stunning, because ROS also participate in the genesis of postischemic dysfunction in xanthine oxidase–deficient species. The exact mechanism whereby ROS depress contractile function remains to be determined (Fig 1A). The number of potential molecular targets is vast because ROS can attack nonspecifically virtually all cellular components. ROS have been shown to interfere with a number of ion transport mechanisms in the sarclemma, including Ca2+ ATPase, Na+-K+ ATPase, and the Na+-Ca2+ exchanger, that would result in increased transsarcomembral Ca2+ influx and cellular Ca2+ overload. Transient exposure to ROS has also been found to decrease the responsiveness of myofilaments to Ca2+ and to impair the function of sarcoplasmic reticulum. Investigation of the manner in which ROS perturb Ca2+ homeostasis and/or Ca2+ responsiveness is important not only because it should elucidate the pathogenesis of stunning but also because it may reconcile the oxyradical and Ca2+ hypotheses of stunning into one pathogenic mechanism (Fig 1A).

Availability of Activator Ca2+

In the setting of global ischemia in vitro, myocardial stunning is not caused by decreased availability of activator Ca2+.
Figure 1. A, Illustration of the proposed pathogenesis of postischemic myocardial dysfunction. This proposal integrates and reconciles different mechanisms into a unifying pathogenetic hypothesis. Transient reversible ischemia followed by reperfusion could result in increased production of superoxide radicals ($\cdot O_2^-$) through several mechanisms, including the following: (1) increased activity of xanthine oxidases; (2) activation of neutrophils; (3) activation of the arachidonate cascade; (4) accumulation of reducing equivalents during oxygen deprivation; (5) derangements of the mitochondrial electron transport system resulting in increased univalent reduction of oxygen; and (6) autoxidation of catecholamines and other substances. Superoxide dismutase (SOD) dismutates $\cdot O_2^-$ to hydrogen peroxide ($H_2O_2$); in the presence of catalytic iron, $\cdot O_2^-$ and $H_2O_2$ interact in a Haber-Weiss reaction to generate the hydroxyl radical ($\cdot OH$). $H_2O_2$ can also generate $\cdot OH$ in the absence of $\cdot O_2^-$ through a Fenton reaction provided that other substances (such as ascorbate) reduce Fe(III) to Fe(II). $\cdot O_2^-$ and $\cdot OH$ attack proteins and polyunsaturated fatty acids, causing enzyme inactivation and lipid peroxidation, respectively. In the setting of reversible ischemia, the intensity of this damage is not sufficient to cause cell death but is sufficient to produce dysfunction of key cellular organelles. Postulated targets of free radical damage include the following: (1) the sarcolemma, with consequent loss of selective permeability, impairment of calcium-stimulated ATPase activity and calcium transport out of the cell, and impairment of the Na$^+$-K$^+$-ATPase activity; the net result of these perturbations would be increased transsarcolemmal calcium influx and cellular calcium overload; (2) the sarcoplasmic reticulum, with consequent impairment of calcium-stimulated ATPase activity and calcium transport; this would result in decreased calcium sequestration (which would contribute to increase free cytosolic calcium) and decreased calcium release during systole (which would cause excitation-contraction uncoupling); and (3) other structures, such as the extracellular collagen matrix (with consequent loss of mechanical coupling) or the contractile proteins (with consequent decreased responsiveness to calcium); for example, free radicals could oxidize thiol groups in myofilaments, causing impaired Ca$^{2+}$ sensitivity. At the same time, reversible ischemia/reperfusion could cause cellular Na$^+$ overload due to (1) inhibition of sarcolemmal Na$^+$-K$^+$-ATPase and (2) acidosis and Na$^+$-H$^+$ exchange. This could further exacerbate calcium overload via increased Na$^+$-Ca$^{2+}$ exchange. An increase in free cytosolic calcium would activate protein kinases, phospholipases, and other degradative enzymes and further exacerbate the injury to the aforementioned key subcellular structures (sarcolemma, sarcoplasmic reticulum, and contractile proteins). Thus, calcium overload could serve to amplify the damage initiated by oxygen radicals. In addition, calcium overload could in itself impair contractile performance and contribute to mechanical dysfunction. It is also possible that the increase in free cytosolic calcium could increase oxyradical production by promoting the conversion of xanthine dehydrogenase to xanthine oxidase. The ultimate consequence of this complex series of perturbations is a reversible depression of contractility. Reproduced with permission of the American Heart Association from Bolli (Circulation. 1990;82:723–738).

B, Possible components of postischemic myocardial dysfunction. Myocardial stunning probably arises from the additive effects of a reperfusion-induced pathology (identified, as least in part, by the fraction [dark shading] of the contractile deficit, which can be restored through the use of an antioxidant intervention given transiently at the time of reperfusion) and a second component (light shading), which incorporates the ischemic pathology from which the heart is slowly recovering, together with any additional reperfusion-induced component that is not amenable to the chosen intervention. Reproduced with permission, Hearse DG. Stunning: a radical review. In: Opie LH, ed. Stunning, Hibernation, and Calcium in Myocardial Ischemia and Reperfusion. Boston, Mass: Kluwer Academic Press; 1992:10–55.
Measurements of free cytosolic \([\text{Ca}^{2+}]\) (using gated NMR, aequorin, or fura 2) in this setting have shown the \(\text{Ca}^{2+}\) transients to be normal or even increased in stunned myocardium.\(^{13,60,64}\) Although \(\text{Ca}^{2+}\) transport has been found to be impaired in sarcoplasmic reticulum isolated from an in vivo model of stunned myocardium,\(^{62}\) no data are available regarding \(\text{Ca}^{2+}\) transients in stunned myocardium in vivo because there is currently no technique that enables measurements of free cytosolic \([\text{Ca}^{2+}]\) in this situation.

**Ca\(^{2+}\) Overload**

On the other hand, there is considerable evidence that a transient \(\text{Ca}^{2+}\) overload during the early phase of reperfusion contributes to the pathogenesis of myocardial stunning after global ischemia in vitro\(^{14,17,28,60}\) (Fig 1A). Indirect evidence indicates that \(\text{Ca}^{2+}\) overload also contributes to posts ischemic dysfunction after regional ischemia in vivo\(^{63}\) but direct demonstration of this concept is still lacking. The mechanism whereby \(\text{Ca}^{2+}\) overload causes contractile dysfunction remains unknown.

**Decreased Sensitivity of Myofilaments to Ca\(^{2+}\)**

Studies in models of stunning after global ischemia in vitro\(^{14,15}\) and in ventricular trabeculae isolated from these models\(^{61}\) have concluded that the alteration responsible for the contractile dysfunction consists of a decrease both in the maximal \(\text{Ca}^{2+}\)-activated force and the sensitivity of myofilaments to \(\text{Ca}^{2+}\). A decrease in \(\text{Ca}^{2+}\) sensitivity of myofilaments has not been observed consistently in all models of global ischemia in vitro, however.\(^{69}\) Studies in single myocytes obtained from a porcine model of myocardial stunning caused by reversible ischemia in vivo have demonstrated that the \(\text{Ca}^{2+}\) sensitivity of tension was markedly decreased with no resolvable change in maximal tension.\(^{64}\) Interestingly, this decrease in \(\text{Ca}^{2+}\) sensitivity occurs after reperfusion, not during ischemia, further supporting the notion that stunned myocardium is a manifestation of reperfusion injury.\(^{65}\) This decrease in \(\text{Ca}^{2+}\) sensitivity of tension appears to be due at least in part to alterations in the cardiac troponin regulatory complex.\(^{66}\) The loss of \(\text{Ca}^{2+}\) sensitivity, however, is not a universal feature of stunned myocardium in this porcine model, because it was observed after a severe ischemic insult but not after milder insults.\(^{67}\) Nevertheless, cells from such “mildly” ischemic regions do display abnormally slow crossbridge cycling, indicative of myofilament injury.\(^{67}\)

If stunned myocardium is due to a decrease in \(\text{Ca}^{2+}\) sensitivity, it is clear that this reduced sensitivity can be overcome. A variety of inotropic agents can stimulate the stunned myocardium to contract, and it is clear that stunned myocardium manifests contractile reserve.\(^{68,70}\) This is good news in the clinical realm because it means that stunned myocardium can be recruited to contract if needed.

**Summary**

It appears that one of the lesions responsible for myocardial stunning after global ischemia in vitro and regional reversible ischemia in vivo is an alteration of the contractile proteins resulting in decreased responsiveness of the contractile machinery to \(\text{Ca}^{2+}\) (Fig 1A), so that for any given \(\text{Ca}^{2+}\) transient, the myocardium generates less force. In this sense, myocardial stunning could be viewed as a disturbance of myofilament function. However, it is not yet clear whether or not myofilament alterations are a general feature of all forms of stunning.

The exact nature of the myofibrillar defect is an important area for future investigation. It has been hypothesized that stunning is the result of the activation of endogenous proteases that attack the myofilaments and that the slow recovery of the stunned myocardium is caused by the slow synthesis of new contractile proteins to repair the damage.\(^{28,71}\) In particular, partial proteolysis of troponin I has been demonstrated in globally stunned myocardium.\(^{66}\) The impaired responsiveness of contractile filaments may also be caused by oxidation of critical thiol groups, which could be quickly reversed on normalization of the cellular redox state\(^2\) (Fig 1A).

**Repetitive Stunning Versus Hibernation**

A major unresolved issue that has important pathophysiological and clinical implications is whether repetitive episodes of myocardial stunning can account for at least some of the clinical manifestations of so-called myocardial hibernation (ie, the syndrome of reversible ventricular dysfunction thought to be secondary to a primary deficit of coronary flow and initially referred to as “chronic stunning”).\(^1\) Animal studies have shown that repeated brief bouts of ischemia have a cumulative effect on contractility, such that the duration and severity of myocardial stunning greatly exceed those induced by a single ischemic episode.\(^7,9,25,33,72,73\) If regional perfusion is not measured simultaneously with regional contractile function, the protracted but ultimately reversible dysfunction associated with repetitive stunning may mimic myocardial hibernation.\(^74\) With repetitive stunning, regional perfusion should recover while function remains depressed. With hibernation, as originally conceived, regional perfusion should be depressed and function should be depressed. Clinically, it is well known that many patients with coronary artery disease experience recurrent episodes of ischemia in the same territory, which may occur on a daily basis, so that the myocardium may remain reversibly depressed for extended periods of time. It is therefore possible that in some clinical cases in which reversible left ventricular dysfunction is thought to be secondary to hibernation, the depression of contractility is in fact secondary to repetitive episodes of stunning.\(^74\)

**Effect of Age on Myocardial Stunning**

In response to brief ischemia, myocardium from senescent animals is more susceptible to myocardial stunning and \(\text{Ca}^{2+}\) overload than that of young animals.\(^75,76\) The mechanism for this increased sensitivity to stunning and impaired ability to maintain \(\text{Ca}^{2+}\) homeostasis is uncertain and may be related to the accumulation of \(\text{Ca}^{2+}\) in the cytosol and nucleus, resulting in DNA nicking associated with endonuclease activation.\(^77\) Whether these changes impair the synthesis of new contractile proteins to repair damaged myofilaments during the recovery phase of stunning is unknown and requires further investigation.

**Myocardial Stunning in Humans**

Myocardial stunning undoubtedly occurs in patients with coronary artery disease in a variety of situations in which the
myocardium is exposed to transient ischemia, such as unstable angina, exercise-induced ischemia, acute myocardial infarction with early reperfusion, open heart surgery, and cardiac transplantation (as reviewed in References 74 and 78-80).

One clinical situation in which stunning has been observed is that of thrombolytic therapy. Several studies that measured global and/or regional left ventricular function did not observe initial dramatic improvement in function after thrombolytic therapy. Improvement in ventricular function occurred gradually over the course of a few weeks.81-84 One report85 suggested that stunned myocardium could be recruited after thrombolytic therapy with small boluses of inotropes. These studies suggest that the ultimate return of cardiac function cannot be determined immediately after reperfusion. Assessment of function a few weeks after thrombolysis will provide a clearer picture as to whether salvaged tissue has recovered.

Stunned myocardium has been observed in patients undergoing cardiac surgery. Despite the modern cardioprotective techniques, some postsurgical stunning is common.86-89 Many patients require inotropic support and pressors for hours to days after surgery, which eventually can be weaned as the stunning abates.

Stunned myocardium occurs after exercise-induced ischemia in patients. Persistent wall-motion abnormalities were observed by echocardiography in patients with severe multivessel coronary artery disease at a time when chest pain and ST-segment deviations had resolved.90 Ambrosio et al91 observed that persistent poststress regional wall-motion abnormalities were present at a time when regional perfusion had recovered. This finding of a flow-function dissociation is strong evidence in support of stunning as the mechanism for this postexercise left ventricular dysfunction. Fraga et al92 showed that diastolic abnormalities could persist in some patients for a few days after an exercise test.

Angioplasty is a useful model for studying the heart’s response to transient episodes of brief ischemia. Brief coronary occlusions (<60 seconds) induced at the time of angioplasty have been associated with persistent abnormalities in ventricular compliance.93 Sheiban et al94 induced longer periods of coronary artery occlusion (5 to 7 minutes) during angioplasty and observed that recovery of regional wall motion required 24 to 36 hours. Recovery in function was improved with calcium channel blockade.94 These findings parallel the experimental observations described in animal models of brief coronary occlusion and reperfusion.

Stunning has been observed by echocardiography in patients with unstable angina.95 In one study,96 regional wall-motion abnormalities were observed immediately after angina and for at least a few hours after chest pain and ECG abnormalities had resolved.

An important clinical implication of stunning is that the phenomenon may contribute to heart failure. Some have postulated that stunning may play a role in ischemic cardiomyopathy.

However, the exact prevalence of this stunned myocardium in humans is unknown because of the difficulty in distinguishing myocardial stunning from other forms of reversible left ventricular dysfunction, such as those caused by silent ischemia and myocardial hibernation. A conclusive diagnosis of myocardial stunning (as opposed to silent ischemia or hibernation) requires simultaneous measurements of regional myocardial perfusion and function, which are rarely available.97 Even when available, techniques for measuring regional perfusion in humans do not have sufficient resolution to discern transmural gradients of myocardial perfusion. This is a major limitation because subendocardial perfusion is the main determinant of transmural contractile function. Another confounding factor is that the coexistence of a subendocardial infarction with subepicardial stunning could be mistaken for hibernation because average transmural perfusion would be decreased.

The clinical situation is likely to be extremely complex, because recurrent bouts of severe ischemia (frequently silent) could be superimposed on a baseline of decreased perfusion, resulting in the superimposition of repetitive stunning on a baseline state of chronic hibernation.74 In many patients with a severe coronary stenosis, the same myocardial region may contain an admixture of subendocardial scar and subendocardial viable myocardium in which stunning and hibernation can both occur, either in close temporal proximity or even simultaneously.98

Future Directions
The members of the workshop identified a number of crucial areas for future investigation that can be grouped into two broad categories: experimental studies and clinical studies.

Experimental Studies
There is a need to integrate further the oxyradical and calcium hypotheses of myocardial stunning and also to augment understanding of the long-term consequences of brief ischemic episodes that cause stunning. Therefore, in experimental models, it will be important to accomplish the following:

1. To identify the precise myofilament lesion responsible for stunning, the mechanism that produces this lesion, and the mechanism whereby this lesion leads to impaired responsiveness to Ca2+ and to contractile dysfunction. Identification of the molecular basis of myocardial stunning would not only advance our understanding of the pathophysiology of ischemic heart disease but would also result in better preventive therapies.

2. To elucidate the role of Ca2+ overload in the genesis of myocardial stunning after regional ischemia (completely reversible or partly reversible ischemia) in vivo and after exercise-induced ischemia. If Ca2+ overload is causally involved in stunning, therapies aimed at preventing the rise in [Ca2+] should be developed.

3. To identify the source(s) of the ROS responsible for stunning, the relative contributions of different ROS to this phenomenon, and the precise mechanism whereby a short burst of ROS production results in prolonged depression of contractile function. Insights into these issues may result in the development of more efficacious antioxidant therapies.

4. To determine the mechanism of myocardial stunning after prolonged, partly reversible regional ischemia (subendocardial infarction) and after exercise-induced ischemia.
The mechanism for stunning in these settings is presently unknown.

5. To develop experimental models of repetitive stunning and chronic hibernation. Use of these models should enable determination of whether repetitive stunning can be a cause of chronic left ventricular dysfunction mimicking hibernation and whether hibernation, defined as a primary decrease in coronary flow, does exist as a chronic condition.

6. To characterize the changes in gene regulation and receptor expression and function associated with stunning. It is now clear that brief, reversible ischemia has a significant effect on gene expression that is likely to result in significant phenotypic changes, particularly after repetitive bouts of ischemia. Given the fact that most patients with coronary disease have recurrent ischemic episodes, these studies would have great clinical relevance.

7. To elucidate the mechanism for the increased susceptibility of aged myocardium to stunning and to characterize the pathophysiology and pathogenesis of stunning in senescent hearts.

**Human Studies**

There is a need to transfer experimental concepts to the clinical setting. Therefore, in human studies it will be important to accomplish the following:

1. To elucidate the pathogenesis of stunning, particularly with regard to the role of myofilament dysfunction and oxidative stress. Little or nothing is known regarding the mechanism of stunning in patients. These studies should translate experimental findings to the clinical arena by testing in humans the pathogenetic hypotheses developed in animal models.

2. To develop better methods for the diagnosis of stunning in humans, including the development of diagnostic techniques that can rapidly distinguish stunning from necrosis after cardiac surgery and after revascularization for acute myocardial infarction. In these situations, such a distinction is essential for selecting the proper management of patients with severe left ventricular dysfunction or cardiogenic shock. Better diagnostic methods that can distinguish stunning from hibernation would also help to define the prevalence, natural history, and clinical importance of these two conditions; ideally, these diagnostic methods should be accurate, relatively inexpensive, and broadly available.

3. To define the natural history of disease progression, especially in the case of repetitive and exercise-induced stunning. Longitudinal follow-up of patients with these syndromes, coupled with serial measurements of regional myocardial function and perfusion, could elucidate whether recurrent or exercise-induced ischemia can result in persistent impairment of ventricular function simulating hibernation.

4. To develop efficacious treatments that can prevent or alleviate myocardial stunning. For the most part, the information acquired in experimental settings regarding the treatment and prevention of myocardial stunning has not yet been translated into clinically applicable therapies. Treatments aimed specifically at preventing myocardial stunning have not been developed in patients. These studies should clarify the usefulness of agents shown to be effective in experimental models (eg, antioxidants, adenosine, K\textsubscript{ATP} channel openers, and angiotensin-converting enzyme inhibitors) as preventive measures against myocardial stunning in such clinical settings as acute infarction with early revascularization, unstable angina, bypass surgery, and cardiac transplantation. Agents that are given during ischemia or at the time of reperfusion might prevent stunning. Other agents (infusion of inotropes) may improve function of the myocardium once it has been stunned; such therapy may be temporary and lasts only as long as the inotrope is infused. Inotropes given to myocardium that appears stunned may worsen ischemia in other areas of the heart if reperfusion or revascularization has not been complete.

**Hibernating Myocardium**

**The Concept of Hibernation**

There is a subset of patients in whom left ventricular function improves significantly after coronary revascularization procedures. Such improvement can occur even in patients with chronic stable angina in whom there was no evidence of active ischemia at the time of revascularization. The functional improvement cannot simply be due to prevention of ongoing necrosis. These clinical observations have led to the concept that myocardium may adapt to chronic ischemia by decreasing its contractility, matching the reduced perfusion with reduced energy demand and thereby preserving viability (Fig 2A). If the ischemia is relieved, the myocardium regains normal contractility. From the earliest days of coronary artery bypass grafting, it was reported that impaired ventricular function was improved by operation. In 1974, in studies at Brigham and Women’s Hospital, Horn et al pointed out that in patients with coronary artery disease and chronic asynergy, epinephrine infusion improved impaired regional wall motion. Postextrasystolic potentiation and exercise also induced increases in contractility. Diamond et al in 1978 that “reports of sometimes dramatic improvement in segmental left ventricular function following coronary bypass surgery, although not universal, leaves the clear implication that ischemic noninfarcted myocardium can exist in a state of function(al) hibernation.” Rahimtoola aptly referred to hibernating myocardium as the persistently impaired function of viable myocardium in the setting of reduced coronary blood flow. In clinical studies, he observed progressive improvement in left ventricular wall-motion abnormalities.
after coronary artery bypass grafting in the absence of transmural necrosis. The concept of hibernating myocardium has stimulated extensive clinical and basic investigation.

**Clinical Observations**

There are numerous clinical studies that support the concept of hibernating myocardium. These studies show that left ventricular wall-motion abnormalities in patients with chronic angina were reversed by successful revascularization. In a classic case report, Rahimtoola reported a patient with single-vessel coronary artery disease (occluded left anterior descending artery) who had a left ventricular ejection fraction of 37\% and a large anteroapical region of akinesis on ventriculography. There had been no history of myocardial infarction. After administration of nitroglycerin, the regional wall-motion abnormality improved substantially. Eight months after coronary artery bypass surgery, the vein graft was patent, the regional wall-motion abnormality had fully resolved, and the ejection fraction had risen to 76\%. Rankin et al showed that 34\% of patients with chronic stable angina had improvement in left ventricular function after coronary artery bypass surgery. From 7 to 14 days after operation, there was an increase in global ejection fraction from 53\% to 71\%. Tillisch et al showed with PET that they could identify areas of reduced perfusion that were metabolically active. After surgical revascularization, the majority of these segments recovered function. Cohen et al identified 12 patients with severe wall-motion abnormalities who had evidence of reversible ischemia and had improvements in global as well as regional wall motion immediately after PTCA.

**Time Course of Recovery of Hibernating Myocardium**

The time course of recovery of hibernating myocardium after revascularization has been variable. One study by Topol et al described nearly immediate improvement in regional wall-motion abnormalities after coronary artery bypass surgery. They used transesophageal echocardiography at the time of cardiac surgery to track changes in left ventricular function. Of 152 analyzed segments, there was an immediate improvement in systolic wall thickening from a prerevascularization value of 43\% to a value of 52\% (\(P < .001\)) after revascularization. As noted above, Cohen et al also observed immediate recovery of function after PTCA. Nienaber et al observed improvements in left ventricular function after PTCA. They studied the effect of PTCA using PET scanning and two-dimensional echocardiography before PTCA, within 72 hours of revascularization, and late (67 to 68 days) after PTCA. Early postangioplasty images showed improvements in regional perfusion assessed by \(^1\)N-ammonia PET imaging. This was not accompanied by an early improvement in wall motion. However, segmental wall motion markedly improved on the late echocardiogram. This study suggested that although hibernating myocardium did recover function after revascularization, such recovery was not necessarily immediate. Baker et al described the case of a patient who had evidence of hibernating myocardium due to compression of the coronary arteries by a ventricular pseudoaneurysm. Severe left ventricular dysfunction was documented by both radionuclide ventriculography and echocardiography. Thallium tomography revealed a perfusion defect in the anterior and anteroseptal distribution of the left ventricle. After repair of the pseudoaneurysm and relief of compression on the coronary arteries, both regional and global function gradually returned toward normal over a 10-week period. One year after repair, thallium imaging showed normal perfusion and echocardiography showed normal function.

Thus, the recovery time of hibernating myocardium after revascularization has been variable. Immediate recovery of function might indicate that hibernation was acute, slow recovery over days to weeks might suggest subacute hibernation, and very slow recovery (months to years) might suggest that the hibernation was chronic. Recovery time could be dependent on a number of factors, including duration of ischemia, severity of ischemia, degree of revascularization (complete versus partial), and amount of myocyte dedifferentiation within the hibernating zone. Recovery that occurs over days to weeks after revascularization of a hibernating segment might represent stunning. Recovery that occurs over a longer time period might represent the regeneration of myofibrils and repair of structural alterations that occurred during the chronic hibernating phase.

Studies described above and many others support the concept of hibernating myocardium as originally defined. However, one limitation of these clinical studies is that in general they do not track reduced flow and reduced function in a chronic manner, that is, at multiple time points before revascularization. Usually, they describe an acute “snapshot” view of the heart before revascularization. The terms “chronic flow reduction” and “chronic wall-motion abnormality” are often used in the description of hibernation, but it is not clear whether the wall-motion abnormalities and reductions of flow are truly chronic in all cases. Also, several PET studies in patients with left ventricular dysfunction observed relatively normal regional coronary blood flow in hibernating segments. Other groups have observed normal oxidative metabolism in these regions, suggesting that oxygen delivery to hibernating segments actually may be normal. One area of active debate is whether hibernating myocardium in patients truly is characterized by reduced flow or normal flow with reduced coronary vasodilator reserve. Some of this debate may be related to the wide variation in what is reported as “normal” coronary flow in humans.

**Detecting Hibernating Myocardium**

Clinical experience has led to acceptance of the idea that hibernation is not only a “real” phenomenon but that it is also of compelling practical significance, at least in a subset of patients with critical coronary lesions. Much effort has gone into developing strategies to assess myocardial viability with a view to identifying those patients who are most likely to benefit from aggressive revascularization. PET has led the way in this regard and has provided insight into the pathophysiology of the adaptation. In some studies, hibernating myocardium exhibits a characteristic switch from aerobic
to anaerobic metabolism, as gauged by enhanced fluorodeoxyglucose uptake in the face of reduced blood flow detected by PET. Such myocardial segments tend to recover function after revascularization.143

Other, more commonly available methods, notably thallium redistribution scintigraphy and dobutamine stress echocardiography, will probably turn out to have similar predictive accuracies.144,145 Thallium redistribution scintigraphy relies on the reasoning that coronary perfusion, although decreased, persists in the hibernating region; furthermore, the hibernating cells maintain membrane integrity and ion homeostasis so that they can accumulate the thallium. Tissue that does not accumulate thallium even with prolonged redistribution or after a secondary thallium infusion is not likely to be viable.146,147

Figure 3. Light and electron microscopy of chronic hibernating myocardium. a and b, Light micrographs of moderately (a) and severely (b) altered myocardial areas. Red stain represents glycogen, which fills the myolytic cytosol. Note the more abundant glycogen stores in the severely affected cells and the accompanying increase in extracellular space (ES) in b. (Magnification ×675.) c and d, Corresponding electron micrographs showing detailed structural remodeling in a moderately (c) and a severely (d) myolytic cardiomyocyte (CM). Glycogen (gl) is abundant; sarcomeres (sm) are present at the cell periphery; mitochondria (m) vary in size and shape (some are very small; arrows); the heterochromatin is uniformly distributed in the nucleus (n); and sarcoplasmic reticulum is virtually absent. These are characteristics of dedifferentiation. (a, magnification ×8.820; b, ×7.650.) Reproduced with permission from M. Borgers, Janssen Research Foundation, Beerse, Belgium.
Dobutamine stress echocardiography assesses the contractile function of various myocardial segments. A region of the heart that functions poorly at baseline may not respond to dobutamine infusion at all (in which case it is nonviable) or may improve or worsen its contractility with the pressor challenge. Myocardium whose function improves with low-level dobutamine infusion but deteriorates with high-level infusion can be expected to improve with revascularization. If this indeed reflects the prototypical response of hibernating myocardium (as is commonly assumed), then hibernating myocardium retains both viability and the ability to respond to inotropic challenges.

More invasive approaches (eg, tissue sampling) are impractical clinically but yield additional clues as to the nature of the adaptation. Human hibernating myocardium has a distinctive histological appearance with myolysis, glycogen accumulation, and increased interstitial fibrosis (Fig 3). It has been suggested that these histopathological changes may represent a dedifferentiation of the myocytes. When advanced, such changes indicate a poor prognosis for recovery even with adequate revascularization.

**Variable Chronicity of Hibernation**

The original clinical descriptions of hibernating myocardium were based on the long-term recovery of function after revascularization (assessed several weeks or months after coronary artery bypass grafting). More recently, the variable chronicity of hibernation has been highlighted, leading to the hypothesis that the longer the ischemia, the slower the subsequent recovery. Virtually all the animal models of hibernation have investigated the myocardial response to acute, low-grade ischemia, a situation that may or may not have common clinical correlates. The observation period is limited to several hours or days at most. In such models, there is a downregulation of contractile function roughly commensurate with the decrease in flow. Stunning generally ensues on the relief of the ischemia, complicating the interpretation of the recovery time course. Such acute compensation to low-grade ischemia is not universally observed and, when it does occur, appears to be operative over a very limited perfusion range. Nevertheless, the pathophysiology of such acute low-flow ischemia is consistent with the teleological notion that hibernating myocardium can downregulate its function (and thus its energy demand) so as to offset the otherwise lethal consequences of limited perfusion (Fig 2A).

**The Concept of Metabolic Adaptation**

There is debate as to whether the hibernating myocardium is truly ischemic or not. Some of this debate hinges on the definition of ischemia itself. If one defines the abnormality of contractility as a crucial component of the definition of ischemia, then hibernating tissue would be considered by some to be truly ischemic. However, if one defines ischemia as a reduction in blood flow severe enough to induce anaerobic metabolism, then hibernating tissue may not be truly ischemic, because there is evidence that hibernating myocardium has undergone a metabolic adaptation. Examples supporting the metabolic adaptation or metabolic “down-regulation” theory of hibernating myocardium come from experimental studies. Animal models of short-term hibernation have shown that after placement of a partial coronary artery stenosis, there is a decrease in contractile function that correlates with a reduction in flow. Myocardial creatine phosphate content decreases during the first few minutes of ischemia induced by partial occlusion but then returns toward normal within 60 to 85 minutes. Fedele et al showed that within 5 minutes after placement of a partial stenosis in an animal model of acute hibernation (in which necrosis did not occur), there was a significant reduction in anterior interventricular vein pH. However, by 180 minutes after stenosis placement, the pH had recovered to normal. In the same study, lactate consumption reversed to lactate production at 5 minutes after stenosis but then recovered toward lactate consumption by 120 minutes after stenosis. Thus, in the setting of ongoing hypoperfusion and contractile dysfunction, the myocardium remained viable and no longer appeared to be metabolically anaerobic. These experimental findings differ somewhat from PET studies in humans, which in some cases have shown increased fluorodeoxyglucose uptake in decreased zones of perfusion, suggesting continued anaerobic metabolism.

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Hibernation Versus Repetitive Stunning

Longer-term models of coronary stenosis have yielded results that suggest a fundamentally different mechanism for hibernation. The central concept here is as follows: chronic coronary artery stenosis acts primarily to limit coronary reserve, not to decrease resting blood flow. Episodes of spontaneous excitement and exercise then lead to repetitive cycles of ischemia precipitated by increased demand in the setting of limited flow reserve. When the ischemia is relieved, the myocardium remains dysfunctional despite having normal basal perfusion; essentially, it is chronically stunned. These observations have led to the proposal that hibernation is the result of repetitive cycles of ischemia and reperfusion (Fig 2B). In this view, cumulative stunning, not downregulation of function to counter chronic low-grade ischemia, leads to the distinctive phenotype of hibernating myocardium. There was considerable discussion related to this area at the workshop, and there were differences of opinion. Some investigators pointed out that there are a wealth of clinical examples and series of patients in which there is evidence of reduced blood flow at rest associated with regional dysfunction, both of which improve after revascularization. It is possible that there are subsets of patients who exhibit true hibernating (with reduced flow), some who exhibit repetitive stunning, and possibly some who exhibit both. This is clearly an area that will require additional research.

Future Directions

The basic biology of hibernation is less understood than either of the other conditions, ie, stunning and preconditioning.
ing; there is no universally accepted animal model, and thus the fundamental mechanisms remain largely unexplained. The situation here is exactly the opposite to that with stunning and preconditioning, both of which were laboratory-based discoveries that subsequently found clinical application. Hibernation, instead, is a clinical phenomenon of potentially immense importance that has proven difficult to study at the basic level.

Given these considerations, the members of the workshop recommended that future effort be concentrated on two areas. The first is to correct the deficiency of appropriate animal models for hibernating myocardium. Although it is recognized that no single animal model may be appropriate to address all of the questions relevant to hibernation, desirable features of such models include the following:

1. **Validation of the model by generally accepted clinical criteria for hibernating myocardium:** PET verification of increased glycolytic precursor uptake relative to blood flow, responsiveness to dobutamine, verification of the characteristic histological features, and demonstration of reversibility with reflow.166

2. ** Exploration of the roles of multivessel disease and/or superimposed atherosclerosis in hibernation.** This recommendation reflects the growing recognition that coronary atherosclerosis may have functional consequences that transcend those attributable solely to simple flow limitation.

3. ** Generation of appropriate small animal models of hibernation with high reproducibility.**

4. **Development of cellular models of hibernation.** Such models could be used to investigate the pathogenesis of the histological lesions, including dedifferentiation, and of the metabolic adaptation, notably the switch from aerobic to anaerobic metabolism.

The second area deemed worthy of further focused study was in the clinical arena, in which several crucial issues regarding natural history and treatment remain to be explored.

The following questions were highlighted as worthy subjects for future investigation:

1. **What is the natural history of hibernating myocardium?** Clinical studies are needed that look at more than just a snapshot view of perfusion and function at one time point before revascularization. Regional function, regional perfusion, and regional viability at multiple time points before revascularization are necessary to answer the question of whether segments of the ventricle truly hibernate as originally conceptualized.

2. **What are the most effective (and most cost-effective) diagnostic and therapeutic strategies?** Might medical therapy be a viable alternative to mechanical revascularization in some patients?

3. **What is the relationship of hibernating myocardium to heart failure?** Does hibernating myocardium commonly underlie ischemic cardiomyopathy? How many segments of hibernating myocardium must be revascularized to substantially improve myocardial function?

4. **Is there any benefit of revascularization of hibernating myocardium independent of the functional improvement?**

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**Preconditioning**

**Biology of Preconditioning**

The term preconditioning was applied to the observation made in 1986 by Murry et al168 that canine myocardium subjected to four brief episodes of ischemia and reperfusion would tolerate a more prolonged episode of ischemia better than myocardium not previously exposed to ischemia. Since that seminal observation in dogs, brief episodes of preconditioning ischemia consistently have been shown to reduce the size of experimentally induced myocardial infarction in rats, rabbits, pigs, and other species. Data from clinical studies suggest that preconditioning probably occurs in humans.169 There is general agreement that other than early reperfusion, preconditioning is the strongest form of in vivo protection against myocardial ischemic injury.

There are certain limits to the benefits of preconditioning. The protection afforded by brief episodes of ischemia is transient and dissipates after 1 to 2 hours of reperfusion. In addition, if the duration of prolonged ischemia is very long (>90 minutes in some models), then the benefits of preconditioning are lost.170 Of note is the observation that in some studies using rabbits, dogs, and rats, the benefits of preconditioning reappear when the interval between preconditioning ischemia and the prolonged ischemic episode is extended to 24 to 72 hours.171-174 A signature of preconditioned myocardium in the canine model is slowed ATP depletion and lactate accumulation during the sustained episode of ischemia.175 This characteristic of slowed energy utilization by preconditioned tissue may be related to the mechanism whereby ischemic preconditioning delays cell death. Murry et al176 pointed out that preconditioned tissue also exhibits a high creatine phosphate concentration, more intracellular glucose, less glycogen, and a smaller adenine nucleotide pool, contains more K+, and is somewhat edematous.

Although the definition of ischemic preconditioning was initially used to describe reduction in myocardial necrosis by brief episodes of preceding ischemia, the definition was extended to include protection against arrhythmias and postischemic left ventricular dysfunction (stunning).177,178 However, the efficacy of ischemic preconditioning in reducing arrhythmias and stunning has not been as consistent as its ability to reduce necrosis. Furthermore, it is unknown whether the protection afforded by preconditioning against arrhythmia and stunning is mediated by the same mechanism that mediates its protection against lethal cell injury.179,180

Another term that has appeared in the literature related to preconditioning is “pharmacological preconditioning.”181,182 This term refers to the ability of pharmacological agents given before coronary occlusion to reduce myocardial infarct size by stimulating the second messenger pathways thought to be involved in preconditioning, but without inducing ischemia. Examples of pharmacological preconditioning include administration of adenosine A1 agonists and K<sub>ATP</sub> channel openers. These agents have also been referred to as “preconditioning mimetics.”169

**Mechanism of Preconditioning**

Determination of the exact mechanism of ischemic preconditioning is important because if the mechanism can be
elucidated, then better therapies (preconditioning mimetics) may be developed to treat a host of ischemic syndromes.

**Role of Protein Kinase C**

Downey and colleagues\(^1\) have proposed that in the rabbit, the mechanism of preconditioning involves stimulation of adenosine, bradykinin, and opioid receptors, which all couple through phospholipases to activate PKC, which then phosphorylates an unknown effector. Specifically, it was initially proposed that translocation of PKC from the cytosol (where it was inactive) to cellular membranes (where it became activated) constituted the memory for preconditioning.

A schematic of the cell signaling pathways followed during preconditioning is shown in Fig 4, as proposed by Downey. Adenosine receptors couple to G\(_i\) protein, which stimulates phospholipase (probably phospholipase D)\(^1\), this in turn degrades membrane phospholipids to phosphatidic acid, which is converted to DAGs; the latter then activates PKC, which eventually phosphorylates some unknown effector, perhaps the sarcolemmal K\(_{\text{ATP}}\) channel. Once the K\(_{\text{ATP}}\) channel is activated, K\(^+\) exits the cells acting as a regional “cardioplegic”; the duration of action potential shortens, reducing energy demand. Studies supporting these hypotheses come from a series of experiments showing that in some models, antagonists of these various steps can block preconditioning, whereas agonists could mimic preconditioning\(^1\)\(^-\)\(^4\). For example, the adenosine A\(_1\) receptor agonist R(-)-N\(_6\)-(2-phe- nylisopropyl)-adenosine (PIA) reduced infarct size in the rabbit model in several laboratories\(^1\)\(^-\)\(^4\). The adenosine antagonist 8-p-sulfophenyl theophylline (SPT) blocked preconditioning. PKC blockers such as phorbol esters and DAGs. Recent studies suggested that free radicals, which directly stimulate phospholipases in cells, also contribute to triggering preconditioning in the rabbit model\(^1\)\(^-\)\(^3\). Evidence also suggests that at least one tyrosine kinase that also may be involved in the mechanism of preconditioning exists downstream from PKC in the rabbit heart\(^1\).

Similar findings were observed in a model of isolated rabbit cardiomyocytes developed by Armstrong et al.\(^1\)\(^\text{a}\)\(^-\)\(^2\). They assessed myocyte injury as the rate of development of osmotic fragility during prolonged ischemic pelleting of cells incubated under oil. Protection of the cells was induced, after a brief ischemic preincubation\(^1\)\(^\text{b}\)\(^-\)\(^2\), by adenosine, adenosine receptor agonists, bradykinin, angiotensin, and phenylephrine. Protection also occurred when the cells were preincubated with the phorbol ester PMA and the PKC activator ingenol. In addition, adenosine receptor antagonists, adenosine deaminase, and selective PKC inhibitors blocked the benefit of ischemic preconditioning.

**Other Cellular Mediators**

Although activation and translocation of PKC is perhaps the most popular and attractive theory to explain infarct size reduction with preconditioning, there are other hypotheses that have been put forward. One of these, advocated by Kitakaze and colleagues,\(^1\) involves activation of 5’-nucleotidase, the enzyme responsible for dephosphorylation of AMP to form adenosine, which would then be protective. A second, alternate hypothesis is that calcium influx during the preconditioning stimulus, perhaps via Na\(^+\)-H\(^+\) and Na\(^+\)-Ca\(^{2+}\) exchange, is important in eliciting cardioprotection.

There is, however, controversy regarding virtually all aspects of the proposed cellular mediators of preconditioning downstream from the initial stimulation of G protein–coupled PKC such as phorbol esters and DAGs.
receptors. For example, Przyklenk et al.\textsuperscript{209,210} concluded that activation of 5′-nucleotidase is not required to elicit cardioprotection, whereas Ovize and colleagues\textsuperscript{201} reported that pharmacological inhibition of tyrosine kinase limited infarct size in control myocardium but failed to block the protective effects of preconditioning. Similarly, Shipolini et al.\textsuperscript{202} failed to confirm the concept that Na\textsuperscript{+}-H\textsuperscript{+} exchange might play a role in preconditioning-induced protection.

Arguments For and Against the Role of PKC

Without doubt, the most hotly debated potential mediator of preconditioning is PKC. Evidence in support of the PKC hypothesis was largely derived from administration of PKC agonists and antagonists in the rabbit and rat models. However, PKC inhibitors failed to attenuate preconditioning in the dog\textsuperscript{203} and pig,\textsuperscript{204,205} whereas brief preischemic infusion of PMA did not limit infarct size in the porcine heart.\textsuperscript{206} When biochemical quantitation as well as fluorescence confocal microscopy was used, PKC translocation was not observed during brief episodes of preconditioning in the canine heart.\textsuperscript{203} However, translocation was observed with longer coronary occlusions and with PMA injections.

Although these observations in the canine and porcine models argue against the role of PKC in ischemic preconditioning, interpretation of both pharmacological and biochemical results may be confounded by limitations in methodology; ie, few PKC inhibitors are selective for PKC alone, and if only one of the 12 isoenzymes of PKC identified to date is important in eliciting protection, measurement of total PKC activity may not reflect the subtle alterations in the subcellular distribution of the one crucial isoenzyme. It is even possible that unidentified receptors may exist in dog or pig that enter the signal transduction pathway downstream of PKC, bypassing it. Quantitative assessment of the activity and distribution of PKC isoenzymes in response to both pharmacological manipulation and ischemic preconditioning per se may be required to resolve this controversy conclusively. In this regard, recent studies have documented isoform-selective translocation of PKC in response to preconditioning stimuli,\textsuperscript{206–208} in some instances with no change in total PKC activity.\textsuperscript{206} Isoform-selective translocation of PKC-δ was observed in isolated rat hearts (immunohistochemistry\textsuperscript{208}), of PKC-α and -ε in isolated rat neonatal cardiomyocytes,\textsuperscript{207} and of PKC-ε and -η with no change in the subcellular distribution of total PKC activity in conscious rabbits (Western immunoblotting).\textsuperscript{206} suggesting that the mechanism of preconditioning might involve selective activation of one or few isozymes. However, even the conclusive identification of isoform-selective PKC translocation may be unable to definitively resolve the controversy because the isoform-specific antibodies often used in these studies are limited by their inability to distinguish whether the isoform is active or inactive.\textsuperscript{209}

Another emerging concept that may be relevant to the mechanism of ischemic preconditioning is that protein phosphatase inhibition appears to protect isolated rabbit cardiomyocytes even when added late in the prolonged ischemic phase.\textsuperscript{210}

Role of Potassium Channels

A final proposed mediator of infarct-size reduction is the ATP-sensitive potassium channel (K\textsubscript{ATP}). Specifically, Gross and colleagues\textsuperscript{211,212} have shown that the K\textsubscript{ATP} channel is important for preconditioning in the canine model. Intravenous glibenclamide, a K\textsubscript{ATP} channel antagonist, given either before or after a single 5-minute period of ischemic preconditioning, abolished the protective effect afforded by preconditioning in reducing infarct size in the canine model.\textsuperscript{211} The nonsulfonylurea K\textsubscript{ATP} channel blocker sodium 5-hydroxydecanoate (5-HD) antagonized the protective effect of preconditioning without affecting infarct size in nonpreconditioned hearts.\textsuperscript{212} These investigators have also shown that a number of K\textsubscript{ATP} channel openers (nicorandil, pinacidil, and bimakalin) are capable of mimicking the effects of ischemic preconditioning. Activation of a variety of receptors (adenosine, acetylcholine, bradykinin, and others) that can mimic the cardioprotective effects of ischemic preconditioning may involve the K\textsubscript{ATP} channel, because glibenclamide and 5-HD were shown to block the cardioprotective effects of these agents.\textsuperscript{213,214}

However, not all studies have implicated K\textsubscript{ATP} channels in preconditioning. Some studies in rabbit hearts, isolated rabbit cardiomyocytes, and rats have not confirmed the importance of K\textsubscript{ATP} channels in preconditioning.\textsuperscript{215–217} A recent study\textsuperscript{218} suggested that the K\textsubscript{ATP} channel blocker glibenclamide, which is often used in these models, must be administered well before the coronary occlusion to block the effect of preconditioning. Although an initial hypothesis was that stimulation of K\textsubscript{ATP} channels might work by shortening the action potential duration and slowing the utilization of ATP, not all studies have documented a shortening of the action potential, and the mitochondrial K\textsubscript{ATP} channels possibly play a role.

The exact final effector mechanisms of preconditioning remain to be elucidated. We do not know what factors exactly explain the resistance to ischemia after preconditioning ischemia. The slowed rate of ATP fall observed in preconditioned tissue could be a consequence rather than a mechanism of preconditioning. If it is a mechanism, why is it transient, only lasting for 10 to 20 minutes during ischemia? It remains to be determined whether the protection is due to preservation of energy stores. Alternatively, the brief episodes of ischemia might limit production of oxidants during a subsequent long ischemia, resulting in protection of membranes. Considerable work is needed to better define the end-effector mechanism of ischemic preconditioning.

Second Window

The second window of protection was described by Yellon and Baxter\textsuperscript{219} and Kuzuya et al\textsuperscript{220} as enhanced tolerance to lethal ischemia 24 hours after a preconditioning stimulus of brief repetitive episodes of ischemia.\textsuperscript{215} The phenomenon of late reappearance of the protective effect of preconditioning has been observed in open chest rabbit and dog and chronically instrumented conscious rabbit and rat.\textsuperscript{172,219–221} Recent work suggests that the delayed anti-infarct effect of preconditioning may extend over a period of 1 to 3 days, unlike the early period of protection by classic preconditioning, which is lost within a few hours.\textsuperscript{222} The mechanism of the delayed
effect of preconditioning is under investigation. The time delay may allow activation of genes and expression of new proteins that could play a role in the late protection. It is unlikely that expression of new proteins plays a role in the early protection after ischemic preconditioning. It is possible that an entirely different set of triggers and signaling pathways is important to the second window. Some of the mechanism that are being studied include expression of heat shock protein (HSP70), alterations in the activity of superoxide dismutase, induction of inducible nitric oxide synthase, and involvement of PKC.\textsuperscript{221,223–225} Recently, Boli et al showed that brief episodes of ischemic preconditioning can protect the porcine and rabbit heart from an episode of stunning 24 hours or more after the preconditioning episode\textsuperscript{226} and that this protection is triggered by the generation of oxyradicals\textsuperscript{227} and nitric oxide.\textsuperscript{228} In addition, they demonstrated that in the conscious rabbit, the delayed protection against infarction is also triggered by the generation of nitric oxide during the initial preconditioning ischemia.\textsuperscript{229} This “second window” of protection against infarction is not, however, seen in the pig model.\textsuperscript{230}

### Stunning Versus Preconditioning

Stunning and preconditioning have similarities in that both phenomena involve brief episodes of ischemia followed by reperfusion. One initial thought regarding the mechanism of preconditioning was that the myocardium, having been stunned by a brief period of ischemia, would have reduced contractility and hence reduced oxygen demand, rendering it more resistant to subsequent ischemia. This concept turned out to be incorrect. First, the myocardial oxygen demand of stunned myocardium has not been reduced in most studies.\textsuperscript{231} Also, Murry et al\textsuperscript{232} studied the temporal relationship between preconditioning and stunning. They observed that one 15-minute coronary occlusion separated by 5 minutes of reperfusion markedly reduced infarct size induced by a 40-minute occlusion. If the reperfusion phase between the brief preconditioning ischemia and 40-minute occlusion was extended to 120 minutes, the myocardium remained severely stunned after the brief ischemia, but the myocardial infarct size plotted against collateral flow returned toward nonpreconditioned values. In another study, Matsuda et al\textsuperscript{233} showed that dobutamine could be used to reverse stunning induced by four 5-minute coronary occlusions in the dog model but that reversing stunning did not prevent preconditioning. Conversely, as already described in regional models of coronary occlusion/reperfusion, classic ischemic preconditioning does not reduce acute stunning.\textsuperscript{159}

Therefore, although brief periods of ischemia induce both stunning and preconditioning, the two phenomena can be dissociated, and preconditioning is not due to simply reduced contractile function of stunned myocardium.

### Clinical Evidence for Preconditioning

The most direct evidence that human myocardium can be preconditioned comes from studies performed in isolated, cultured human cardiomyocytes subjected to simulated ischemia and reperfusion. Ikonomidis et al\textsuperscript{234} showed that prior brief episodes of ischemia improved survival when cultured human cardiac myocytes were exposed to 90 minutes of sustained ischemia. Studies of human myocardium obtained at the time of cardiac surgery have demonstrated better preservation of ATP during 10-minute periods of aortic cross-clamp fibrillation when hearts were exposed to prior 3-minute episodes of ischemia.\textsuperscript{235} In addition, in the same model, it has recently been shown that troponin release is significantly attenuated in patients who had been preconditioned previously.\textsuperscript{236} Studies using human cardiac muscle (atrial trabeculae) confirm findings in the animal laboratory with respect to initiation of preconditioning with hypoxia and the adenosine A\textsubscript{1} agonist rPIA.\textsuperscript{237} Furthermore, the signaling pathways in human cardiac muscle appear to involve PKC, with supporting evidence for the role of the $K_{ATP}$ channel as a positive end effector.\textsuperscript{238}

Repeated coronary artery occlusions in the course of PTCA may simulate the preconditioning phenomenon. Several clinical studies have observed progressive decreases in chest pain, ST-segment elevation, and lactate production on subsequent compared with first 60- to 90-second intracoronary balloon inflation.\textsuperscript{239,240} The potassium channel blocker glibenclamide can prevent the beneficial effects of repeated balloon angioplasty. Adenosine can mimic the protective effect,\textsuperscript{241} whereas adenosine agonists block it,\textsuperscript{242,243} findings that parallel experimental observations in the animal laboratory. Some studies that assessed collateral flow showed no evidence of recruitment during sequential balloon inflations,\textsuperscript{239} although one study\textsuperscript{240} did report that in about half of the patients, there was recruitment.

A number of clinical studies have shown that preinfarct angina may confer benefit in acute myocardial infarction, including smaller infarct size assessed by enzymes or ECG, better in-hospital survival, less in-hospital heart failure and shock, better left ventricular function, and lower rate of arrhythmia.\textsuperscript{244–246} In the recent TIMI 9 trial,\textsuperscript{247} preinfarction angina reduced the combined end point of 30-day mortality, heart failure, and recurrent myocardial infarction, but only when the preinfarct angina occurred within 24 hours of infarction. Ishihara et al\textsuperscript{248} showed that patients with prodromal angina within 24 hours of infarction had an improved 5-year survival. Although ischemic preconditioning is one potential mechanism whereby preinfarct angina confers benefits to infarct patients, other potential mechanisms include earlier and more complete thrombolysis, which has been observed in one study\textsuperscript{249} and may imply a preconditioning-like effect on the vasculature, and the development of intramural collaterals that are not detected by coronary angiography.

Patients with coronary artery disease have been observed to exercise longer before developing angina and may develop less angina and ischemia during a second exercise test compared with a first test when these tests are separated by a brief rest period.\textsuperscript{250,251} The clinical observation of the “warm-up” phenomenon may represent one aspect of preconditioning in humans.

Mentzer\textsuperscript{252} and colleagues have applied the pharmacological preconditioning concept to patients undergoing open heart surgery. Placebo and various doses of adenosine (100 $\mu$mol/L, 1 mmol/L, 2 mmol/L, and 2 mmol/L plus a
6-minute 140 μg · kg⁻¹ · min⁻¹ pretreatment infusion) were administered to patients before cardiopulmonary bypass. Patients were hemodynamically monitored before, during, and after dosing, before being placed on cardiopulmonary bypass, after weaning from bypass, and then for 24 hours. Transesophageal echocardiography was performed before bypass, after bypass, and during the postoperative stay. The results showed that adenosine was safe and well tolerated in the doses used. The patients receiving high-dose adenosine required significantly less or no vasoactive drugs during the first 24 hours after heart surgery and had improved average regional wall motion by echocardiography. This is one of the first trials to apply pharmacological preconditioning directly to humans to try to improve outcomes. There have been anecdotal reports of actually inducing ischemic preconditioning (by transiently clamping a coronary artery and then reperfusing) before minimally invasive directed coronary artery bypass.

Future Directions

The members of the workshop suggested studies within three general areas: mechanism of preconditioning, the biology of preconditioning, and clinical studies.

Mechanism of Preconditioning

If preconditioning mimetic pharmaceuticals are going to be developed for the clinical treatment of ischemia, then it is crucial to learn the mechanisms of preconditioning.

1. There is a need to develop models allowing assessment of intracellular signaling pathways in preconditioning and determination of the final effector(s). These models will require multidisciplinary approaches and should be designed to help resolve the roles of suggested signaling pathways, including but not limited to PKC, 5'-nucleotidase, adenosine A₁ receptors, and K<sub>ATP</sub> channels. Small-animal models (ie, rabbit) may rely on PKC-dependent signaling, whereas large animals (dog, pig) may rely on K<sub>ATP</sub> and other mechanisms that may be independent of PKC.

2. There is a need to clarify the mechanism of the second window (or late phase) of ischemic preconditioning. Very little is known regarding the mechanism of the second window.

3. Because it is unknown which mechanisms are most important in humans, it would be prudent to include whole animal models of both small and large animals. Isolated myocyte models in humans and in large and small animals should also be developed.

Biology of Preconditioning

There is a need to characterize further the short-term and long-term “biology” of preconditioning. With the use of large- and small-animal models and cell-based models, studies should be specifically designed to accomplish the following:

1. Ascertain whether metabolic changes (such as slower rate of ATP degradation and attenuation of acidosis) and improvement in cell volume regulation are mere markers of preconditioning or are necessary for protection.

2. Determine whether preconditioning is effective or altered in aged and diseased hearts.

3. Establish the long-term consequences of preconditioning on left ventricular remodeling, infarct expansion, and survival.

4. Elucidate the properties of the “second window of protection,” particularly in large-animal models.

5. Determine whether preconditioning exerts a beneficial effect on arrhythmias and the vasculature, including the recent concept that a preconditioned vessel may be more amenable to thrombolysis.

Clinical Studies of Preconditioning

There is a need for well-designed clinical trials to test ischemic preconditioning agonists (or mimetics) such as adenosine, A<sub>1</sub> and A<sub>3</sub> agonists, and/or K<sub>ATP</sub> channel openers, administered either acutely before an ischemic event or 24 hours before planned ischemia (mimicking the second window of protection). Suggested clinical arenas for studies should include (1) angioplasty (a useful setting for initial screening of potential mimetics), (2) coronary artery bypass grafting and other open heart surgery, (3) minimally invasive directed coronary artery bypass (MIDCAB), (4) preconditioning of a donor transplant heart, and (5) unstable and stable angina.

Summary

During the last 15 years, the concepts of stunning, hibernation, and preconditioning have emerged as new phenomena that relate to myocardial ischemia/reperfusion. Stunning and hibernation have the potential to contribute to heart failure, and clinicians are becoming increasingly aware of these phenomena. In particular, preconditioning has the potential to lead to new therapies for a variety of ischemic syndromes. The participants at this NHLBI workshop strove to consider numerous factors in producing a list of promising yet practical opportunities to advance our understanding of these ischemic conditions. The recommendations emerging from the workshop are designed as a guide to the NHLBI’s Heart Research Program in planning support of studies addressing stunning, hibernation, and preconditioning and their roles in ischemia, heart failure, and arrhythmia. It is hoped that future efforts in this area will ultimately improve the national cardiovascular health.

Appendix

Participants in the Workshop

Eugene Braunwald, MD, Workshop Chairman, Brigham and Women’s Hospital, Harvard Medical School, Boston, Mass; Leslie J. Reinlib, PhD, Workshop Coordinator, National Heart, Lung, and Blood Institute, Bethesda, Md; Roberto Bolli, MD, Workshop Co-Chairman, University of Louisville, Louisville, Ky; Robert A. Kloner, MD, PhD, Workshop Co-Chairman, University of Southern California, Good Samaritan Hospital, Los Angeles; Eduardo Marban, MD, PhD, Workshop Co-Chairman, Johns Hopkins University, Baltimore, Md; Robert O. Bonow, MD, Northwestern University Medical School, Chicago, Ill; Marcel Borgers, PhD, Janssen Research Foundation, Beerse, Belgium; James Downey, PhD, University of South Alabama, Mobile; Harvey Feigenbaum, MD, Indiana University, Indianapolis; Charles Ganote, MD, Quillen College of Medicine, Johnson City, Tenn; Garrett Gross, PhD, Medical College of Wisconsin, Milwaukee; Robert Jennings, MD, Duke University Medical Center, Durham, NC; Sidney Levitsky, MD, Harvard Medical School, Boston, Mass; James D. McCully,
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PhD, Harvard Medical School, Boston, Mass; Robert Mentzer, Jr, MD, University of Kentucky Medical Center, Lexington; William P. Miller, MD, University of Wisconsin, Madison; Karin Przyklenk, PhD, Good Samaritan Hospital, Los Angeles, Calif; Shahabudin Rahimtoola, MD,* University of Southern California, Los Angeles; Stephen Vatner, MD, Allegheny University of the Health Sciences, Pittsburgh, Pa; and Derek Yellon, DSc, HomMRCP, FESC, The Hatter Institute, University College London Hospital and Medical School, London, United Kingdom.

*Participated in workshop; elected not to participate in preparation of manuscript.

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