Mechanism of Myocardial “Stunning”

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Postischemic ventricular dysfunction, or myocardial “stunning,” has recently become the focus of considerable attention among researchers and clinicians. There are two fundamental reasons for the explosive growth of interest in this phenomenon. First, it is now recognized that spontaneous reperfusion after coronary spasm or thrombosis is common in patients with coronary artery disease. This implies that postischemic myocardial stunning is likely to be an important component of the natural history of this disorder. Second, with the advent of interventional recanalization, an increasingly large number of patients are subjected to coronary reperfusion in an effort to preserve left ventricular function. The occurrence of myocardial stunning in these patients could significantly delay the benefits of reperfusion therapy.

During the past decade, the efforts of experimental investigators have produced considerable progress in our understanding of the mechanism responsible for postischemic contractile dysfunction. This is clearly the central problem because it will not be possible to effectively prevent myocardial stunning unless its pathogenesis is elucidated. The purpose of this article is to critically review the various mechanisms proposed for postischemic dysfunction and their pathophysiological and clinical implications. The major unresolved issues and areas for future research will be identified, and an attempt will be made to integrate different theories into a unifying pathogenetic paradigm.

Definition of Myocardial Stunning

One cannot overemphasize the importance of a clear definition of myocardial stunning because this term is sometimes loosely applied to situations in which the persistence of contractile abnormalities in postischemic tissue is due to other causes (e.g., myocellular death). Postischemic dysfunction, or myocardial stunning, is the mechanical dysfunction that persists after reperfusion despite the absence of irreversible damage. The essential point of this definition is that postischemic dysfunction, no matter how severe or prolonged, is a fully reversible abnormality. Diagnosis of myocardial stunning should not be made unless reasonable assurance can be provided that the tissue in question is still entirely viable.

In accordance with this definition, myocardial stunning is a relatively mild, sublethal injury that must be kept quite distinct from myocardial infarction. It is unknown whether these two conditions share a common mechanism. Consequently, this review will discuss only results obtained in experimental preparations in which mechanical dysfunction is likely to be due to stunning rather than to cell death. The mechanism of lethal ischemia-reperfusion injury has been reviewed previously and is beyond the scope of this article.

Classification of Myocardial Stunning

Experimentally, the concept of myocardial stunning encompasses a wide variety of settings with major pathophysiological differences. A classification is in order because different mechanisms have been described in different experimental conditions. The available observations can be grouped into the following categories (Table 1).

Myocardial Stunning After One Completely Reversible Ischemic Episode

In the dog, a coronary occlusion lasting less than 20 minutes does not result in any myocardial necrosis but nevertheless produces prolonged abnormalities of contractile performance. This is the classic model of myocardial stunning, the one in which the phenomenon was originally described, and the one most commonly used in experimental investigations.

Myocardial Stunning After Multiple Completely Reversible Ischemic Episodes

Repeated brief (5 or 10 minutes) coronary occlusions have a cumulative effect on systolic function and result in prolonged contractile impairment despite absence of irreversible damage. The largest decrease in contractility occurs after the first occlusion; with subsequent occlusions, the additional decrements in mechanical performance become progressively smaller. (Whether repetitive ischemia also has a cumulative effect on the time to complete recovery, however, remains unclear.) This model of
myocardial stunning differs from the single 10- or 15-minute occlusion model in that the mechanical dysfunction develops gradually and is associated with a considerably greater total ischemic burden (50–60 versus 10–15 minutes).

**Myocardial Stunning After One Partly Irreversible Ischemic Episode (Subendocardial Infarction)**

In the dog, when reperfusion is instituted after a period of coronary occlusion of more than 20 minutes but less than 3 hours, the subendocardial portion of the region at risk is generally found to be infarcted, whereas variable quantities of subepicardial tissue remain viable.\(^2\) The recovery of function in this subendocardial region salvaged by reperfusion is, however, delayed for days or weeks.\(^8,21-25\) It is particularly difficult to evaluate the effect of therapy on this form of postischemic dysfunction because the reperfused region contains a complex admixture of necrotic subendocardium and stunned subepicardium, and the relative proportions of these two components are highly variable. Other confounding factors include the tethering of surviving myocytes by dead, nonfunctional tissue; the expansion of the infarcted region; and the progressive replacement of necrotic myocardium by scar.

**Myocardial Stunning After Global Ischemia in Isolated Hearts**

The occurrence of cell death in these preparations depends on species, temperature, duration of ischemia, and perfusate composition. Under selected conditions, isolated hearts reperfused after transient ischemia exhibit complete normalization of phosphocreatine content and intracellular pH,\(^26-34\) indicating that viability is generally preserved. Although in these models the reversibility of the contractile abnormalities cannot be verified, the metabolic recovery suggests that the injury is mostly nonlethal. Accordingly, despite the numerous obvious differences from ischemia in vivo, under selected circumstances isolated heart preparations can mimic myocardial stunning. In other cases, however, these preparations may be associated with significant cell death,\(^35-44\) and their relevance to myocardial stunning is questionable.

**Myocardial Stunning After Global Ischemia During Cardioplegic Arrest In Vivo**

Despite the use of hypothermic cardioplegia, global ischemia in intact animals is usually followed by prolonged contractile abnormalities.\(^45-48\) The reversibility of these derangements has not been documented, but under carefully controlled conditions they are likely to be due mostly to stunning.

**Myocardial Stunning After Exercise-Induced Ischemia**

Stresses such as exercise provoke myocardial ischemia and dysfunction in animals with a flow-limiting coronary stenosis. With cessation of exercise, these contractile abnormalities persist.\(^49\) Thus, myocardial stunning can also occur after high-flow ischemia, in which the primary problem is an increase in oxygen requirements rather than a decrease in supply.

Because of the many significant pathophysiological differences among these situations, one cannot assume that observations made in one setting necessarily apply to the others. An important, unresolved issue is whether all forms of stunning share a common pathogenesis.

**Factors That Determine the Severity of Myocardial Stunning**

In conscious dogs undergoing a 15-minute coronary occlusion, there is a sensitive coupling between the degree of myocardial dysfunction after reperfu-
TABLE 2. Mechanisms Proposed for Myocardial Stunning

Most likely mechanisms
- Generation of oxygen-derived free radicals
- Excitation-contraction uncoupling due to sarcoplasmic reticulum dysfunction
- Calcium overload

Other proposed mechanisms
- Insufficient energy production by mitochondria
- Impaired energy use by myofibrils
- Impairment of sympathetic neural responsiveness
- Impairment of myocardial perfusion
- Damage of the extracellular collagen matrix
- Decreased sensitivity of myofilaments to calcium

sion and the magnitude of blood flow reduction during the preceding period of ischemia, whereby even small differences in ischemic perfusion are associated with large differences in postischemic recovery.12 (In open-chest dogs, this correlation is weaker,50 probably because of the confounding effects of anesthesia and trauma.) Furthermore, the severity of stunning is greater in the inner layers of the left ventricular wall, which are the most severely ischemic, than in the outer layers.14,51 Another important factor is the duration of flow deprivation: The longer the ischemic period, the greater the ensuing mechanical abnormalities.9,52

In conclusion, the severity of postischemic dysfunction is determined primarily by the severity and duration of the antecedent ischemia. This concept has two important implications. First, whatever may be the precise mechanism responsible for stunning, such mechanism must be initiated and modulated by perturbations associated with ischemia. Although stunning appears to be a form of “reperfusion injury” (see below), it is ischemia that “primed” the myocardium for the development of such injury. Second, any intervention that improves perfusion during ischemia would be expected to attenuate stunning after reflow. Reducing the severity of ischemia is probably the most effective way to reduce postischemic dysfunction.

Mechanism of Myocardial Stunning

Thus, in very general terms, the abnormalities of the postischemic myocardium are governed by the abnormalities occurring during ischemia. But what is the specific sequence of events whereby transient ischemia leads to prolonged depression of contractility?

Several different hypotheses, which are not necessarily exclusive, have been proposed (Table 2) and are reviewed below.

Insufficient Energy Production by Mitochondria

ATP levels in the stunned myocardium are depressed and recover slowly,8,24,53–55 with a time course similar to that of contractile function.8,24 Thus, in the early 1980s, the hypothesis was advanced1,54,55 that postischemic dysfunction may result from an inability of the myocardium to resynthesize enough high-energy phosphates to sustain contractile function, possibly because of loss of adenine nucleotide precursors. Numerous subsequent observations, however, have refuted this theory. First, no correlation is observed between myocardial ATP levels and recovery of contractility in several models of postischemic dysfunction.26,27,56–59 Second, the content of phosphocreatine in the stunned myocardium is normal or supranormal (“phosphocreatine overshoot”),8,26,27,30,53,55,57,60 implying that the phosphorylation ability of the mitochondria is intact. Third, the stunned myocardium responds to inotropic stimuli with a marked and sustained increase in contractility, such that function exceeds the levels measured at baseline.17,30,61–66 This striking inotropic response occurs without a decrease in ATP or phosphocreatine stores,30,63 indicating that energy production is not inherently impaired and can keep pace with high metabolic demands. Fourth, manipulations that increase ATP levels in the stunned myocardium fail to increase mechanical function.33,67

In summary, the available evidence suggests that a defect in the rate of resynthesis of ATP by the mitochondria is not the primary cause of myocardial stunning.

Impaired Energy Use by Myofibrils

Myocardial stunning is associated with a decrease in myofibrillar creatine kinase activity68 and a reduction of the substrate (free ADP) used by the myofibrillar creatine kinase to produce ATP at the contraction site.30,68 Both of these abnormalities might disrupt normal energy use and thus have been postulated to contribute to postischemic dysfunction.68 This hypothesis, however, appears implausible because it does not explain the considerable contractile and metabolic reserve of the stunned myocardium.17,30,61–66 Indeed, the reversal of postischemic dysfunction by inotropic stimuli implies that the residual activity of myofibrillar creatine kinase is still sufficient to run the myofibrillar ATPase reaction at normal or supranormal rates.

Impairment of Sympathetic Neural Responsiveness

After 25 minutes of coronary occlusion, the reperfused myocardium loses its inotropic responsiveness to sympathetic nervous stimulation, suggesting injury to the sympathetic-neural axis.69 However, no such defect is observed after 15 minutes of coronary occlusion.70 Furthermore, as noted above, myocardial stunning occurs in isolated (and thereby denervated) hearts. Although functional sympathetic denervation may contribute to postischemic dysfunction in certain situations in which contractility is strongly dependent on sympathetic drive, it seems unlikely that this abnormality plays a major role in most experimental preparations of myocardial stunning.

Impairment of Myocardial Perfusion

When myocardial stunning is produced by a 15-minute coronary occlusion, postreperfusion subepi-
cardiac blood flow is normal, whereas subendocardial flow is slightly decreased (−20%).11–14,71–73 The mechanism and significance of this phenomenon are unclear.72,73 Although it is possible, as speculated by Heyndrickx et al,11 that the reduced subendocardial perfusion may contribute to the impaired mechanical performance, two considerations suggest that it is not a major causative factor. First, there is no correlation between subendocardial blood flow and wall thickening in the postischemic myocardium.72,73 Second, the loss of systolic function (dyskinesia or akinesis) is out of proportion to the slight (−20%) decrease in subendocardial perfusion.72,73

When myocardial stunning is produced by repeated 5-minute coronary occlusions, blood flow to the postischemic myocardium, measured by radioactive microspheres, is generally normal.15–17,19 However, microspectrophotometry reveals increased vein-to-vein heterogeneity of oxygen saturations, with an excess of vessels demonstrating very low oxygen saturations, suggesting that the stunned myocardium contains discrete microregions of either increased oxygen extraction or reduced flow.19 A nonuniform reduction of flow (microregions of ischemia intermixed with microregions of hyperemia) has also been invoked to explain the enhancement of function that is observed in stunned myocardium when perfusion is augmented.16 However, the findings that myocardium stunned by repeated 5-minute coronary occlusions has normal flow reserve16 and intact microvascular patency19 exclude nonuniform ischemia secondary to anatomical obstruction. Furthermore, the hypothesis of persistent ischemia as a cause of dysfunction does not explain the normal or near-normal contractile reserve of the postischemic myocardium.17,65,66

In summary, impaired perfusion (either uniform or nonuniform) is unlikely to be the primary pathogenetic mechanism in postischemic contractile failure.

Damage of Extracellular Collagen Matrix

Using a model of myocardial stunning produced by 12 5-minute coronary occlusions separated by 10 minutes of reperfusion, Eng’s group has demonstrated that postischemic dysfunction is associated with severe ultrastructural damage of the extracellular collagen matrix74 and a modest (−10%) loss of collagen.75 These findings have led to the hypothesis that myocardial stunning may be due to a structural defect, namely, disruption of the mechanical coupling function provided by the extracellular collagen network.74 To fully evaluate this hypothesis, it will be important to explore the time course of the collagen changes and its relation to the time course of postischemic dysfunction. Because in conscious dogs undergoing one brief (<15 minutes) coronary occlusion most of the contractile recovery takes place in the first 3–4 hours of reperfusion9,12,13,52 (an interval too short for significant resynthesis or remodeling of the collagenous components), it seems improbable that structural damage to collagen plays a pathogenetic role in this model of myocardial stunning.

Indeed, a recent study76 demonstrated that collagen is disrupted by 12 5-minute coronary occlusions but not by one 15-minute occlusion followed by reperfusion.

In summary, collagen damage may contribute to myocardial stunning after multiple ischemic episodes but is unlikely to be a causative factor after a single completely reversible ischemic episode (Table 1). One possible explanation for this difference is that in the former setting, repetitive ischemia results in repetitive systolic bulging for a total duration of more than 15 minutes; this may cause greater mechanical stress and, consequently, greater damage of the extracellular collagen network.

Decreased Sensitivity of Myofilaments to Calcium

Using isolated ferret hearts subjected to 15 minutes of global ischemia at 37°C, Kusuoka et al27 observed that the stunned myocardium exhibits decreased responsiveness to calcium, as manifested by a decrease in the maximal calcium-activated force and a decrease in the myocardial sensitivity to extracellular calcium. They speculated that the reduced sensitivity to extracellular calcium could in turn be due to either a decrease in the intracellular free Ca2+ concentration ([Ca2+]i) transient or a decrease in the sensitivity of myofilaments to calcium.27 The former possibility seems improbable because two recent studies with different techniques32,77 indicate that the calcium transient is (paradoxically) increased in the stunned myocardium after 1532 or 20 minutes77 of global ischemia at 37°C in isolated ferret hearts. Accordingly, it has been proposed32,77 that the fundamental mechanism for postischemic dysfunction is not insufficient availability of free cytosolic calcium during systole but instead is reduced sensitivity of the contractile apparatus to calcium. One problem with this hypothesis derived from in vitro studies is that it does not explain two observations made in vivo. First, when the stunned myocardium is challenged with inotropic stimuli, it exhibits a normal or near-normal contractile reserve.17,65,66 Second, the apparent sensitivity of the stunned myocardium to intracoronary calcium is not decreased.65 If the primary problem was a reduced sensitivity of myofilaments to calcium, then the contractile response to exogenous calcium or to other inotropic agents (which act by raising [Ca2+]i) should be reduced.

In summary, studies in vitro suggest that myocardial stunning is a result of reduced calcium sensitivity rather than reduced calcium availability, but studies in vivo are not readily reconcilable with this interpretation (Table 1).

Calcium Overload

Several lines of evidence point to a pathogenetic role of calcium overload in myocardial stunning. First, when isolated ferret hearts subjected to 15 minutes of global normothermic ischemia are reperfused with solutions containing low concentrations of calcium, the postischemic contractile abnormalities
are significantly attenuated. The results of this experiment, in which no intervention is applied during ischemia, indicate that calcium entry upon reperfusion is an important mechanism of myocardial stunning. A decrease in the severity of stunning is also observed in hearts pretreated with ryanodine, an inhibitor of cellular calcium overload. Second, exposure of isolated ferret hearts to a transient calcium overload in the absence of ischemia produces mechanical and metabolic abnormalities similar to myocardial stunning. Third, transient intracellular acidosis during early reperfusion can prevent myocardial stunning in isolated ferret hearts subjected to 15 minutes of global ischemia and open-chest dogs undergoing 15 minutes of coronary occlusion. Because acidosis antagonizes not only the influx of calcium into cells (by inhibiting the Na+-Ca2+ exchange and the slow calcium channels) but also the intracellular binding of calcium, these results provide indirect evidence that a transient calcium overload contributes to postischemic dysfunction. Fourth, recent studies have shown that [Ca], increases between 10 and 20 minutes of global ischemia in isolated hearts. This is the same duration of ischemia that results in myocardial stunning. In these models, [Ca], returns to normal values within a few minutes after reperfusion but appears to remain transiently elevated during very early reflow.

The mechanism for this rise in [Ca], during ischemia remains uncertain. It may be secondary to decreased calcium uptake by the sarcoplasmic reticulum. It may also be mediated through the Na+-Ca2+ exchange, secondary to a rise in intracellular Na+ during ischemia due to 1) metabolic inhibition of the Na+,K+-ATPase, and 2) acidosis and consequent Na+H+ exchange. Because the Na+-Ca2+ exchange is inhibited by acidosis and intracellular pH in the stunned myocardium normalizes quickly after reflow, entry of calcium via the Na+-Ca2+ exchange may be greatly increased upon reperfusion. Further research will be necessary to identify the precise circumstances leading to calcium overload. The mechanisms by which a transient calcium overload induces prolonged contractile dysfunction are also unclear. Increased cytosolic calcium can activate phospholipases and other degradative enzymes. In addition, elevation of cytosolic calcium could trigger production of oxygen radicals via xanthine oxidase (see below).

The calcium overload theory is not incompatible with the fact that exogenous calcium ameliorates function in the stunned myocardium. The increase in [Ca], is postulated to be a brief phenomenon immediately after reflow, following which there may be either a normalization of [Ca], transients or a relative calcium deficiency (see “Excitation-Contraction Uncoupling Due to Sarcoplasmic Reticulum Dysfunction”). The early excessive [Ca], levels could damage intracellular organelles concerned with contraction and thereby produce prolonged mechanical dysfunction.

In summary, considerable evidence suggests that a transient calcium overload during early reperfusion contributes to the pathogenesis of myocardial stunning after global ischemia in vitro. The role of calcium overload in vivo models of myocardial stunning remains to be defined (Table 1) and represents an important area for future investigations.

Effect of calcium channel blockers. Calcium channel blockers have been used to gain insights into the role of altered calcium homeostasis in vivo, but unfortunately the results are difficult to interpret. Verapamil, diltiazem, nifedipine, nitrendipine, and amlodipine have been shown to improve recovery of function in regionally stunned myocardium in intact animals. However, it is unclear whether these beneficial effects reflected a direct protective action of the drugs or were mediated by favorable modifications of afterload, preload, heart rate, and regional myocardial blood flow, all of which could change the systolic properties of the stunned myocardium. A direct protective action was demonstrated by a recent study in which nifedipine enhanced the functional recovery of the stunned myocardium independently of any effect on systemic hemodynamics or regional myocardial blood flow. However, interpretation of these results is problematic because the beneficial effects of nifedipine were noted even when treatment was started 30 minutes after reperfusion, whereas myocardial stunning has been suggested to result from a transient calcium overload immediately after reflow. It is unlikely that nifedipine acted in a specific way to decrease [Ca], because administration of calcium or inotropic agents (which raise [Ca],) improves contractile function in the stunned myocardium. The mechanism of action of nifedipine in this intriguing study remains to be elucidated.

Excitation-Contraction Uncoupling Due to Sarcoplasmic Reticulum Dysfunction

Krause et al investigated this mechanism in a canine model of postischemic dysfunction produced by eight to 12 5-minute occlusions separated by 10-minute reflow periods. Sarcoplasmic reticulum isolated from the stunned myocardium demonstrated a decrease in the ability to transport calcium, concomitant with a reduction in the activity of the Ca2+,Mg2+-ATPase. These researchers proposed that a decrease in the amount of calcium stored in the sarcoplasmic reticulum as a result of a reduction in the calcium pump activity could diminish contractile protein activation through attenuated calcium release during systole.

The concept of inadequate delivery of calcium to the contractile proteins, secondary to sarcoplasmic reticulum dysfunction, is an attractive hypothesis. The postulated relative calcium deficiency would be consistent with the observation that exogenous administration of calcium can return contractile function of stunned myocardium to preischemic levels. It would
also be consistent with the notion that inotropic agents (which increase [Ca\textsubscript{i}]) can reverse myocardial stunning.\textsuperscript{17,30,61--66} However, since this hypothesis implies that the amplitude of the [Ca\textsubscript{i}] transient is decreased, it is not easily reconcilable with in vitro data\textsuperscript{52,77} (see above). Considerably more research is necessary to determine whether similar dysfunction of the sarcoplastic reticulum occurs in other models of myocardial stunning (Table 1) and whether it is associated with decreased [Ca\textsubscript{i}] transients in vivo. In addition, the factors that cause injury to the sarcoplastic reticulum remain to be elucidated.

**Generation of Oxygen-Derived Free Radicals**

*Effect of antioxidants on myocardial stunning after a brief coronary occlusion.* Approximately 7 years ago, a number of investigators, including ourselves, postulated that myocardial stunning is caused in part by the generation of reactive oxygen metabolites, such as superoxide anion (·O\textsubscript{2}\textsuperscript{-}), hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), and hydroxyl radical (·OH), and began a series of experiments designed to test this hypothesis. We used an open-chest canine preparation in which the left anterior descending coronary artery is occluded for 15 minutes and then reperfused.\textsuperscript{96} In the first study,\textsuperscript{97} we found that administration of superoxide dismutase (SOD) (an enzyme that catalyzes the dismutation of ·O\textsubscript{2}\textsuperscript{-} to O\textsubscript{2} and H\textsubscript{2}O\textsubscript{2}) and catalase (an enzyme that reduces H\textsubscript{2}O\textsubscript{2} to O\textsubscript{2} and H\textsubscript{2}O) significantly enhanced recovery of function after reperfusion. Attenuation of myocardial stunning by SOD and catalase was also observed by other investigators\textsuperscript{50,57,98} using similar experimental preparations. In a more recent study,\textsuperscript{99} we found that neither SOD nor catalase alone significantly improved recovery of function in the stunned myocardium; however, when the two enzymes were combined, contractile recovery was significantly greater than that observed in controls or in dogs receiving either enzyme alone. These results suggest that both ·O\textsubscript{2}\textsuperscript{-} and H\textsubscript{2}O\textsubscript{2} contribute to the cellular damage responsible for myocardial stunning and that combined administration of SOD and catalase is more likely to be effective against stunning than separate administration. The inability of SOD alone to mitigate postischemic dysfunction has also been observed in a pig model.\textsuperscript{100}

Although both ·O\textsubscript{2}\textsuperscript{-} and H\textsubscript{2}O\textsubscript{2} appear to contribute to stunning, it remains uncertain whether they do so by direct cytotoxicity or via formation of other species. ·O\textsubscript{2}\textsuperscript{-} and H\textsubscript{2}O\textsubscript{2} can interact through the metal-catalyzed Haber-Weiss reaction to generate the highly reactive ·OH radical.\textsuperscript{101} Consequently, accumulation of ·O\textsubscript{2}\textsuperscript{-} and H\textsubscript{2}O\textsubscript{2} could produce postischemic dysfunction, at least in part, indirectly through generation of ·OH. To elucidate this problem, we evaluated dimethylthiourea, an ·OH scavenger that is more effective than traditional ·OH scavengers\textsuperscript{102} and does not react with ·O\textsubscript{2}\textsuperscript{-} or H\textsubscript{2}O\textsubscript{2} in vitro.\textsuperscript{103} We observed that dimethylthiourea produced a significant and sustained improvement in the function of the stunned myocardium.\textsuperscript{103} These results were further corroborated by studies with N-2-mercaptopropionyl glycine (MPG), a free radical scavenger that readily enters the intracellular space and is active orally. MPG was found to be a powerful scavenger of ·OH with no effect on ·O\textsubscript{2}\textsuperscript{-} or H\textsubscript{2}O\textsubscript{2} in vitro\textsuperscript{104} and to effectively attenuate myocardial stunning in vivo.\textsuperscript{104,105} Taken together, these results suggest that the ·OH radical (or one of its reactive products) is a mediator of postischemic dysfunction and that the beneficial effects of SOD and catalase previously demonstrated\textsuperscript{50,57,97--99} are due in part to prevention of ·OH generation. This conclusion is also consistent with the finding that the iron chelator desferrioxamine attenuates postischemic dysfunction.\textsuperscript{106,107} Because iron catalyzes the formation of ·OH (through the Haber-Weiss or Fenton mechanism) as well as the propagation of ·OH-initiated lipid peroxidation,\textsuperscript{101} the protective effects of desferrioxamine are compatible with the view that ·OH is a mediator of myocardial stunning. The effectiveness of antioxidants that scavenge ·OH or prevent its generation should not be construed as evidence that all of the damage initiated by oxygen is mediated via ·OH. It is true that H\textsubscript{2}O\textsubscript{2} is a relatively nonreactive species and its toxicity may be due in large part to its reduction to ·OH.\textsuperscript{101} However, there is evidence that ·O\textsubscript{2}\textsuperscript{-} can directly cause cellular toxicity in vitro (Reference 108; reviewed in Reference 109). Furthermore, because catalase alone prevents formation of ·OH by removing H\textsubscript{2}O\textsubscript{2}, the fact that SOD has to be added to catalase to produce significant attenuation of stunning in vivo\textsuperscript{99} implies the existence of a component of damage specifically due to ·O\textsubscript{2}\textsuperscript{-}. It appears, therefore, that all three species (·O\textsubscript{2}\textsuperscript{-}, H\textsubscript{2}O\textsubscript{2}, and ·OH) are important in postischemic dysfunction (·O\textsubscript{2}\textsuperscript{-} and ·OH as mediators of injury and H\textsubscript{2}O\textsubscript{2} as a precursor of ·OH).\textsuperscript{99}

Although the studies discussed above\textsuperscript{50,57,97--99,103--107} consistently support the oxyradical hypothesis, their significance is limited by the fact that they were all performed in open-chest animals. Thus, artifacts due to the combined effects of anesthesia, hypothermia, surgical trauma, volume and ionic imbalances, nonphysiological conditions, and associated neurohumoral perturbations, as well as other potentially confounding variables, cannot be excluded. It is therefore essential that the oxyradical hypothesis be tested in conscious animal preparations. In a recent experiment,\textsuperscript{110} we observed that the combined administration of SOD and catalase significantly enhanced recovery of function after a 15-minute coronary occlusion in conscious, unsedated dogs and that this effect was sustained for 24 hours after reflow. The accelerated recovery was not followed by any subsequent deterioration, indicating that postischemic depression of contractility is not a useful "protective" response to injury.

In summary, numerous investigations from several independent laboratories\textsuperscript{50,57,97--99,103--107,110} uniformly suggest that oxygen metabolites play a significant role
in the genesis of myocardial stunning after a 15-minute period of ischemia, in both open-chest and conscious animals. The relative contributions of the various metabolites have not been definitively established. It is clear that a portion of the damage is mediated by -OH; however, the available evidence suggests that myocardial stunning cannot be simplistically ascribed to a single oxygen species and that all of the three initial metabolites of oxygen (O₂⁻, H₂O₂, and -OH) contribute to the cellular injury responsible for postischemic dysfunction. Iron also appears to play a role in stunning, presumably by catalyzing the formation of -OH.

**Direct evidence for the oxyradical hypothesis.** A major limitation of the studies reviewed heretofore is that all of the evidence provided by these investigations is indirect and therefore inconclusive. Clearly, to definitively confirm a causative role of oxygen metabolites in postischemic dysfunction, it is necessary to directly demonstrate and quantitate free radical generation in the stunned myocardium in the presence and absence of antioxidant interventions.

Production of free radicals has been directly demonstrated in isolated rabbit or rat hearts undergoing global ischemia and reperfusion, but because of the numerous important differences in experimental conditions, results obtained in vitro cannot necessarily be extrapolated to the intact animal. We used the spin trap α-phenyl N-tert-butyl nitroxide (PBN) and electron paramagnetic resonance (EPR) spectroscopy to detect and measure production of free radicals in our in vivo model of postischemic dysfunction (15-minute coronary occlusion in open-chest dogs).

After infusion of PBN, EPR signals characteristic of PBN radical adducts were detected in the venous blood draining from the ischemic-reperfused vascular bed. The EPR signals were consistent with a mixture of different secondary lipid radicals, such as alkyl and alkoxy radicals. The myocardial production of radicals began during coronary occlusion but increased dramatically after reperfusion, peaking at 2–4 minutes. After this initial burst, production of radicals abated but did not cease, persisting for as long as 3 hours after reflow. There was a linear, positive relation between the magnitude of adduct production and the magnitude of ischemic flow reduction, indicating that the intensity of free radical generation after reflow is determined by the severity of the antecedent ischemia—the greater the degree of hypoperfusion, the greater the subsequent production of free radicals and, by inference, the severity of reperfusion injury. These findings imply that interventions that improve perfusion during ischemia will attenuate free radical reactions after reflow. Generation of free radicals in in vivo models of regional myocardial stunning has also been reported by Leiboff et al.

In a subsequent study, we found that SOD plus catalase suppressed the production of free radicals in the stunned myocardium, indicating that these radicals are derived from univalent reduction of oxygen. Furthermore, the inhibition of free radical production was associated with inhibition of myocardial stunning. More recently, we observed that MPG or desferroxamine administered just before reperfusion markedly attenuated myocardial stunning and the associated production of PBN adducts; however, the same agents given 1 minute after reperfusion did not attenuate myocardial stunning or initial PBN adduct production. Thus, three different antioxidant interventions (SOD plus catalase, MPG, and desferroxamine) reduced postischemic dysfunction at the same doses and under the same experimental conditions in which they reduced formation of PBN adducts. The correlation between the two effects suggests that the production of free radicals in the stunned myocardium plays a causal role in the depression of contractility.

In summary, the results of the studies that have measured free radicals in the stunned myocardium provide direct evidence supporting a pathogenetic role of oxygen metabolites. Specifically, these studies indicate that 1) free radicals are produced in the stunned myocardium in the intact dog after reversible regional ischemia, 2) the univalent pathway of reduction of oxygen is the source of the radicals, and 3) inhibition of free radical reactions results in enhanced recovery of contractility (i.e., the radical reactions are necessary for postischemic dysfunction to occur).

**Effect of oxygen radicals on cardiac function.** The ability of oxygen metabolites to depress myocardial function has been directly demonstrated in vitro and in vivo. Exposure of isolated rabbit interventricular septa, isolated rat or rabbit papillary muscles, and isolated rat or rabbit hearts to free radical-generating solutions or pure H₂O₂ has uniformly resulted in decreased mechanical function and ATP levels (i.e., in changes similar to those observed in the stunned myocardium). In most of the studies in which an attempt was made to discern the relative roles of different oxygen species, it was found that the deleterious effects could be prevented by catalase or by -OH scavengers but not by SOD, suggesting that H₂O₂ or its byproduct, -OH, was the oxygen metabolite responsible for the observed depression of contractile function. In one study, however, SOD was protective, whereas catalase had no effect, implying that the major negative inotropic agent was O₂⁻. These in vitro observations have been recently expanded by Przyklenk et al. who reported that infusion of xanthine oxidase plus purine plus iron-loaded transferrin, administered through a coronary vein in open-chest dogs, resulted in the development of significant wall motion abnormalities.

In summary, it is clear that reactive oxygen species depress myocardial contractility both in vitro and in vivo. The precise role of each species remains to be defined. There is considerable evidence that the detrimental effects of -O₂⁻ and H₂O₂ on cardiac
function are mediated in part by generation of \( \cdot \text{OH} \). This is in accordance with the results of in vivo studies,\textsuperscript{103–107} which suggest an important role of \( \cdot \text{OH} \) as a mediator of stunning. However, there is also evidence for a direct negative inotropic action of \( \cdot \text{O}_2^- \), which is also congruent with observations in vivo.\textsuperscript{99}

**Mechanism of oxyradical-mediated contractile dysfunction.** The precise mechanism whereby oxygen metabolites depress contractile function remains speculative and represents one of the major unresolved issues pertaining to the pathogenesis of myocardial stunning. Free radicals are reactive species that have no specific target and can attack virtually all cellular components. In theory, every abnormality thus far described in the stunned myocardium (see above) could be caused by oxyradicals. At least two key cellular components, proteins and lipids, are likely to be involved in free radical–initiated reactions in the postischemic myocardium. Activated oxygen species can denature proteins and inactivate enzymes.\textsuperscript{126} Furthermore, they can produce peroxidation of the polyunsaturated fatty acids contained in cellular membranes,\textsuperscript{127} which would impair selective membrane permeability and interfere with the function of various cellular organelles.

Evidence for the occurrence of lipid peroxidation in the stunned myocardium is provided by two recent studies. Romaschin et al\textsuperscript{128} observed increased myocardial concentration of hydroxy conjugated dienes (which are products of free fatty acid oxidation) during and after a 45-minute period of global normothermic ischemia in open-chest dogs. The maximal concentration of conjugated dienes was measured at 5 minutes of reflow. Importantly, the tissue examined after reperfusion was dysfunctional but not necrotic, thus representing stunned myocardium. Weisel et al\textsuperscript{129} subsequently reported that in patients undergoing cardioplegic arrest during coronary artery bypass surgery, there was myocardial release of conjugated dienes in the coronary sinus blood at 3 and 60 minutes of reperfusion, which was associated with a decrease in the myocardial concentration of the antioxidant \( \alpha \)-tocopherol.

Dysfunction of the sarcoplasmic reticulum may be an important mechanism whereby oxyradicals mediate the contractile abnormalities observed after reversible ischemia. As mentioned above, both calcium uptake and \( \text{Ca}^{2+},\text{Mg}^{2+} \)-ATPase activity have been found to be significantly depressed in the stunned myocardium.\textsuperscript{95} A similar decrease in calcium uptake and \( \text{Ca}^{2+},\text{Mg}^{2+} \)-ATPase activity is observed in isolated sarcoplasmic reticulum after exposure to oxygen radicals,\textsuperscript{127,130} and oxyradical scavengers preserve sarcoplasmic reticulum function.\textsuperscript{127,130}

Other studies suggest that the sarcolemma may be a critical target of free radical–mediated damage. Oxyradicals have been shown to interfere with calcium transport and calcium-stimulated ATPase activity in the sarcolemma.\textsuperscript{131,132} Oxygen radicals have also been shown to interfere with the \( \text{Na}^{+},\text{K}^{+} \)-\text{Ca}^{2+} exchange\textsuperscript{133} and to inhibit the \( \text{Na}^{+},\text{K}^{+} \)-ATPase activity.\textsuperscript{134} Furthermore, the sarcolemmal \( \text{Na}^{+},\text{K}^{+} \)-ATPase activity is impaired in reperfused myocardium, and this impairment is prevented by antioxidants.\textsuperscript{135} Impairment of the \( \text{Na}^{+},\text{K}^{+} \)-ATPase activity would result in \( \text{Na}^{+} \) overload, with consequent activation of the \( \text{Na}^{+} - \text{Ca}^{2+} \) exchange activity.\textsuperscript{87,88} All of these observations imply that excessive production of oxyradicals could result in increased transsarcolemmal calcium influx and cellular calcium overload.

It is important to point out that the foregoing postulated mechanisms involve alterations in calcium homeostasis and thus would reconcile the oxyradical and calcium-overload hypotheses of stunning into one pathogenetic mechanism.

**Sources of oxygen radicals in the stunned myocardium.** Among the various potential mechanisms of oxyradical generation in the stunned myocardium, only two have been explored thus far, namely, the enzyme xanthine oxidase and the activated neutrophil. We found that the xanthine oxidase inhibitor, allopurinol, produced a marked improvement in the functional recovery of the stunned myocardium.\textsuperscript{136} Furthermore, allopurinol inhibited the increased cardiac production of urate observed in control dogs during ischemia and early reperfusion, indicating effective inhibition of xanthine oxidoreductase.\textsuperscript{136} Attenuation of stunning has also been demonstrated with oxypurinol after 15\textsuperscript{137} and 90 minutes\textsuperscript{138} of ischemia and reperfusion. These data suggest that xanthine oxidase is one of the sources of the oxygen radicals that contribute to postischemic dysfunction in the dog. Whether this concept applies to humans remains uncertain because the myocardial content of xanthine oxidase is species dependent. Reports regarding the myocardial content of the enzyme in the human heart are conflicting.\textsuperscript{139–143}

In contrast to these earlier reports,\textsuperscript{136–138} in a recent study,\textsuperscript{144} oxypurinol and amiflutzole, two xanthine oxidase inhibitors, failed to mitigate stunning after 15 minutes of ischemia in dogs. Whether the discrepancy is due to different degrees of inhibition of xanthine oxidoreductase or to other reasons is unclear.

Leukocytes are another potential source of oxygen metabolites.\textsuperscript{145,146} However, several investigators have found that myocardial stunning is not attenuated by depletion of neutrophils with antisemur71,147 or filtration,\textsuperscript{148} by administration of nafazatrom, which inhibits production of leukotrienes,\textsuperscript{149} by administration of antibodies to the adhesion-promoting Mo1 glycoprotein,\textsuperscript{150} and by administration of dextran, which inhibits leukocyte adherence to the endothelium.\textsuperscript{151} Recent studies have shown that the neutrophil content of the stunned myocardium after a 12-minute period of ischemia is decreased\textsuperscript{152} and that myeloperoxidase activity (a marker of neutrophils) in myocardium stunned by a 15-minute occlusion is not different from nonischemic myocardium.\textsuperscript{150} Furthermore, myocardial stun-
ning occurs in isolated heart preparations that are devoid of circulating neutrophils. Taken together, these results suggest that granulocytes do not play a major role in the genesis of postischemic dysfunction after brief, reversible ischemia, although they appear to be major mediators of irreversible tissue injury and vascular obstruction after prolonged periods of ischemia.145,146,153

In contrast to the aforementioned findings, however, others have reported that depletion of granulocytes by Leukopak filtration prevents or alleviates postischemic dysfunction after a 15-minute coronary occlusion. The reasons for the differences between these studies and the aforementioned investigations are unclear and warrant additional research.156 One possibility to be explored, raised by Engler and Coveill, is that the relation between circulating neutrophil counts and myocardial damage may be nonlinear. In summary, in the canine model of myocardial stunning produced by one reversible ischemic episode, xanthine oxidase may be a source of free radicals (although the data are not entirely consistent), whereas the role of neutrophils remains controversial. Of course, many other processes could lead to formation of \( \mathrm{O}_2^- \) and \( \mathrm{H}_2\mathrm{O}_2 \) during myocardial ischemia and reperfusion, including activation of the arachidonate cascade, autoxidation of catecholamines and other compounds, ischemia-induced damage of the electron transport chain in the mitochondria, and accumulation of reducing equivalents.127 It will be difficult to selectively assess the role of these potentially important mechanisms in a complex system such as the intact animal. Identification of the sources of oxyradicals in the stunned myocardium represents another major unresolved issue.

**Time course of free radical–induced damage in the stunned myocardium.** When exactly in the ischemia-reperfusion sequence that leads to stunning does the crucial free radical–induced damage take place? In a recent study, we observed that infusion of the antioxidant MPG attenuated postischemic dysfunction to a similar extent regardless of whether the infusion was started before ischemia or 1 minute before reperfusion; however, infusion started 1 minute after reflow was ineffective, suggesting that the critical radical-mediated injury occurs in the first few moments of reperfusion. We have subsequently obtained similar results with desferrioxamine. Furthermore, the spin trap PBN enhances contractile recovery even when the infusion is commenced 20 seconds before reflow; the magnitude of the protective effect is similar to that observed when the infusion is commenced before ischemia. Captopril, an angiotensin converting enzyme inhibitor with alleged \( \mathrm{O}_2^- \) scavenging properties, produces similar attenuation of postischemic dysfunction when the administration is started before ischemia or 2 minutes before reperfusion. The conclusion that a substantial portion of the cellular damage responsi-
acetylcysteine attenuate myocardial stunning independently of infarct size limitation in closed-chest dogs subjected to 90 minutes of coronary occlusion and 24 hours of reflow.

Initial observations indicate that SOD and catalase do not alleviate exercise-induced stunning.

In summary, there is strong evidence that oxyradicals contribute to postsischemic dysfunction after global ischemia (in vitro as well as in vivo) and after multiple episodes of regional ischemia. They do not appear to contribute to exercise-induced postsischemic dysfunction. The role of oxygen radicals in myocardial stunning after a prolonged, partly irreversible ischemic insult remains uncertain and represents a major unresolved problem. Elucidation of this issue will be difficult because the dysfunction is due in part to the presence of infarction and in part to the presence of stunning—a situation that complicates the evaluation of therapy, as discussed above.

Integration of Different Hypotheses

Myocardial stunning is probably a multifactorial process that involves complex sequences of cellular perturbations and the interaction of multiple pathogenetic mechanisms. Our understanding of this phenomenon is still fragmentary, and none of the theories discussed herein (Table 2) explains the entire cascade of events that culminates in postsischemic contractile failure. For example, the oxyradical hypothesis does not explain how reactive oxygen species cause mechanical dysfunction, and the sarcoplasmic reticulum theory does not explain why this organelle is damaged. Integration of the various hypotheses is complicated by the fact that for the most part, each hypothesis has been developed in a different experimental preparation (Table 1).

Nevertheless, it is important to emphasize that these hypotheses are not mutually exclusive and in fact may represent different parts of the same pathophysiological sequence. There is considerable evidence to suggest a link between generation of oxygen radicals and impaired calcium homeostasis in the setting of reperfusion injury. For example, the damage associated with the "calcium paradox" resembles that associated with the "oxygen paradox" and probably has a similar pathogenetic mechanism. Furthermore, as discussed above, oxyradicals generated upon reperfusion can cause dysfunction of the sarcoplasmic reticulum and alter calcium flux across the sarcolemma. The result of these actions would be excitation-contraction uncoupling and cellular calcium overload. In this regard, it has recently been demonstrated that reoxygenation of cultured myocytes results in Ca\(^{2+}\) overload, which can be greatly attenuated by antioxidant enzymes. Oxygen radicals could also damage the proteins of the contractile machinery in a manner that reduces their responsiveness to calcium. On the other hand, calcium overload may exaggerate oxyradical production by promoting the conversion of xanthine dehydroge-
Summary

Among the numerous mechanisms proposed for myocardial stunning, three appear to be more plausible: 1) generation of oxygen radicals, 2) calcium overload, and 3) excitation-contraction uncoupling.

First, the evidence for a pathogenetic role of oxygen-derived free radicals in myocardial stunning is overwhelming. In the setting of a single 15-minute coronary occlusion, mitigation of stunning by antioxidants has been reproducibly observed by several independent laboratories. Similar protection has been recently demonstrated in the conscious animal, that is, in the most physiological experimental preparation available. Furthermore, generation of free radicals in the stunned myocardium has been directly demonstrated by spin trapping techniques, and atten-
vation of free radical generation has been repeatedly shown to result in attenuation of contractile dysfunction. Numerous observations suggest that oxyradicals also contribute to stunning in other settings: after global ischemia in vitro, after global ischemia during cardioplegic arrest in vivo, and after multiple brief episodes of regional ischemia in vivo. Compelling evidence indicates that the critical free radical damage occurs in the initial moments of reflow, so that myocardial stunning can be viewed as a sublethal form of oxyradical-mediated "reperfusion injury."

Second, there is also considerable evidence that a transient calcium overload during early reperfusion contributes to postischemic dysfunction in vitro; however, the importance of this mechanism in vivo remains to be defined.

Third, inadequate release of calcium by the sarcoplasmic reticulum, with consequent excitation-contraction uncoupling, may occur after multiple brief episodes of regional ischemia, but its role in other forms of postischemic dysfunction has not been explored.

It is probable that multiple mechanisms contribute to the pathogenesis of myocardial stunning. The three hypotheses outlined above are not mutually exclusive and in fact may represent different steps of the same pathophysiological cascade. Thus, generation of oxyradicals may cause sarcoplasmic reticulum dysfunction, and both of these processes may lead to calcium overload, which in turn could exacerbate the damage initiated by oxygen species. The concepts discussed in this review should provide not only a conceptual framework for further investigation of the pathophysiology of reversible ischemia-reperfusion injury but also a rationale for developing clinically applicable interventions designed to prevent postischemic ventricular dysfunction.

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