

Molecular and Cellular Mechanisms of Myocardial Stunning

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Bolli, Roberto, and Eduardo Marbán. Molecular and Cellular Mechanisms of Myocardial Stunning. *Physiol. Rev.* 79: 609–634, 1999.—The past two decades have witnessed an explosive growth of knowledge regarding postischemic myocardial dysfunction or myocardial “stunning.” The purpose of this review is to summarize current information regarding the pathophysiology and pathogenesis of this phenomenon. Myocardial stunning should not be regarded as a single entity but rather as a “syndrome” that has been observed in a wide variety of experimental settings, which include the following: 1) stunning after a single, completely reversible episode of regional ischemia in vivo; 2) stunning after multiple, completely reversible episodes of regional ischemia in vivo; 3) stunning after a partly reversible episode of regional ischemia in vivo (subendocardial infarction); 4) stunning after global ischemia in vitro; 5) stunning after global ischemia in vivo; and 6) stunning after exercise-induced ischemia (high-flow ischemia). Whether these settings share a common mechanism is unknown. Although the pathogenesis of myocardial stunning has not been definitively established, the two major hypotheses are that it is caused by the generation of oxygen-derived free radicals (oxyradical hypothesis) and by a transient calcium overload (calcium hypothesis) on reperfusion. The final lesion responsible for the contractile depression appears to be a decreased responsiveness of contractile filaments to calcium. Recent evidence suggests that calcium overload may activate calpains, resulting in selective proteolysis of myofibrils; the time required for resynthesis of damaged proteins would explain in part the delayed recovery of function in stunned myocardium. The oxyradical and calcium hypotheses are not mutually exclusive and are likely to represent different facets of the same pathophysiological cascade. For example, increased free radical formation could cause cellular calcium overload, which would damage the contractile apparatus of the myocytes. Free radical generation could also directly alter contractile filaments in a manner that renders them less

responsive to calcium (e.g., oxidation of critical thiol groups). However, it remains unknown whether oxyradicals play a role in all forms of stunning and whether the calcium hypothesis is applicable to stunning in vivo. Nevertheless, it is clear that the lesion responsible for myocardial stunning occurs, at least in part, after reperfusion so that this contractile dysfunction can be viewed, in part, as a form of "reperfusion injury." An important implication of the phenomenon of myocardial stunning is that so-called chronic hibernation may in fact be the result of repetitive episodes of stunning, which have a cumulative effect and cause protracted postischemic dysfunction. A better understanding of myocardial stunning will expand our knowledge of the pathophysiology of myocardial ischemia and provide a rationale for developing new therapeutic strategies designed to prevent postischemic dysfunction in patients.

I. INTRODUCTION

It has been almost 25 years since postischemic myocardial dysfunction was first described by Vatner's group in conscious dogs undergoing brief coronary occlusions followed by reperfusion (61). The term *myocardial stunning* was coined in 1982 (29). At the time of its discovery, this phenomenon received relatively little attention because coronary reperfusion was thought to be a rare occurrence. Myocardial stunning was regarded mostly as a laboratory curiosity. Beginning in the 1980s and continuing to an even greater extent in the 1990s, however, postischemic dysfunction has become the focus of increasing interest both among experimentalists (12) and clinicians (14) for two major reasons. First, coronary reperfusion by means of thrombolytic therapy, percutaneous transluminal coronary angioplasty, or coronary artery bypass graft surgery has become a standard approach to the management of acute ischemic syndromes in patients with coronary artery disease. Second, several studies have demonstrated that many patients experience spontaneous reperfusion as a result of lysis of coronary thrombi or release of coronary spasm. Accordingly, it has become increasingly evident that postischemic myocardial stunning is part of the natural history of coronary artery disease and may contribute significantly to the morbidity associated with this disorder (14).

Although our knowledge regarding myocardial stunning has grown dramatically over the past two decades, much of the available information remains fragmented and linked to specific experimental settings. Myocardial stunning is a heterogeneous entity. Several forms of this contractile abnormality have been described. Although a number of hypotheses appear to explain its mechanism in a given setting, the extent to which these hypotheses are applicable to all forms of stunning and the interrelationships among various mechanisms remain unclear. Thus there is a need to integrate different, seemingly conflicting, concepts pertaining to the genesis of postischemic myocardial dysfunction and to assess their implications from a broad perspective that transcends a specific experimental model.

This review summarizes current knowledge regarding the pathophysiology and pathogenesis of myocardial stunning in a manner that is both comprehensive and critical. Emphasis is placed on general concepts rather than on a detailed analysis of data. Sections II-VI of this review address the definition and pathophysiology of myocardial stunning, particularly with respect to the various experimental models utilized to study this abnormality. In sections VII-IX, the two major hypotheses regarding the mechanism of myocardial stunning (i.e., the "oxyradical hypothesis" and the "calcium hypothesis") are critically reviewed, and a unifying pathogenetic paradigm that integrates both is proposed. Finally, in sections X-XIII, the relationship between reperfusion injury and stunning is addressed, and the major unresolved issues and areas for future research are identified. This review deals primarily with the basic aspects of stunning; the reader is referred elsewhere (14) for a review of the clinical aspects of this phenomenon.

II. HISTORICAL BACKGROUND

The phenomenon of postischemic dysfunction was initially described by Heyndrickx et al. (61) in 1975. These authors reported that regional mechanical function remained depressed for more than 3 h after a 5-min coronary occlusion and for more than 6 h after a 15-min occlusion in conscious dogs. During the subsequent years, other investigators (28, 81, 162) confirmed that the recovery of contractile performance after a brief (~20 min) coronary occlusion is delayed; furthermore, a similar phenomenon was demonstrated after prolonged (1-2 h) coronary occlusion resulting in subendocardial infarction (30, 42, 90, 156). The concept of postischemic dysfunction was expanded further by the observation that contractility remains depressed for variable intervals after selective subendocardial ischemia (102) and exercise-induced ischemia (66) and that repetitive bouts of myocardial stunning can produce a prolonged, reversible depression of contractility that mimics myocardial "hibernation" (137).

TABLE 1. *Classification of myocardial stunning and evidence for the various mechanisms proposed in experimental animals*

Experimental Setting	Evidence for a Pathogenetic Role of			
	Oxygen radicals	Calcium overload	Ca ²⁺ -activated proteases	Reduced calcium sensitivity
<i>Stunning due to decreased blood flow</i>				
Regional ischemia				
Single, completely reversible ischemic episode	++	?	?	-
Multiple, completely reversible ischemic episodes	+	?	?	-
Single, partly irreversible ischemic episode (subendocardial infarction)	±	?	?	?
Global ischemia				
Isolated heart in vitro	+	+	+	+
Cardioplegic arrest in vivo	+	?	?	?
<i>Stunning due to increased O₂ demands</i>				
Exercise-induced ischemia				
In the presence of coronary stenosis	-	?	?	?
In the absence of coronary stenosis (hypertrophy)	?	?	?	?

+, Published studies support this mechanism; ++, published studies from multiple laboratories consistently support this mechanism (evidence is also available in conscious animal preparations); -, published studies do not support this mechanism; ±, published studies are conflicting; ?, no data are available. [Adapted from Bolli (12).]

III. DEFINITION OF MYOCARDIAL STUNNING

One cannot overemphasize the importance of a clear definition of myocardial stunning, for this term is sometimes inappropriately applied to situations in which the persistence of contractile abnormalities in postischemic tissue is due to other causes (such as myocellular death, persistent ischemia, or nonischemic injury). Postischemic dysfunction, or myocardial stunning, is the mechanical dysfunction that persists after reperfusion despite the absence of irreversible damage and despite restoration of normal or near-normal coronary flow (12). The two essential points of this definition are 1) that postischemic dysfunction, no matter how severe or prolonged, is a fully reversible abnormality and 2) that the dysfunction is not caused by a primary deficit of myocardial perfusion (12). Two corollaries follow from this definition. First, in experimental settings the diagnosis of myocardial stunning should not be made unless reasonable assurance can be provided that the tissue in question is still entirely viable and that flow is normal or near-normal (12). Second, in clinical settings the diagnosis of stunning requires demonstration of two major points: 1) that the contractile abnormality is reversible and 2) that the dysfunctional myocardium has normal or near-normal flow (14).

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, and therefore, data obtained in models of infarction should not be extrapolated to models of stunning.

IV. HETEROGENEITY OF MYOCARDIAL STUNNING

Myocardial stunning, as defined above, is not a single entity but rather a syndrome that has been observed in a wide variety of experimental settings with major pathophysiological differences (12). The common denominator to these heterogeneous settings is that in all of them the myocardium is exposed to a transient ischemic episode that is not long enough to cause irreversible injury. Because the heterogeneity of the experimental models of stunning is likely to be associated with heterogeneous pathogenetic and pathophysiological substrates, it is important to discuss briefly the differences among the various settings.

The experimental observations can be classified into the following categories (12) (see sect. IV, A-F, and Table 1).

A. Myocardial Stunning After a Single, Completely Reversible Ischemic Episode

In the dog, a coronary occlusion lasting <20 min does not result in any myocardial necrosis (70), but upon reperfusion, the recovery of contractile performance in the previously ischemic myocardium is delayed for several hours (23, 28, 34, 60, 61, 162). This is the "classic" model of myocardial stunning (12), the one in which the phenomenon was originally described (61), and the one most commonly used in experimental investigations (12). The exact duration of postischemic contractile abnormal-

ities in this model has varied in different experimental preparations. In conscious dogs, the average transmural systolic wall thickening (an integrated measure of function across the ventricular wall) remains depressed up to 24 h after a single 15-min coronary occlusion (28). The rate of recovery, however, is faster in the subepicardium than in the subendocardium, suggesting that stunning is a nonuniform phenomenon that is most severe in the subendocardium (23). Both systolic and diastolic function are depressed in stunned myocardium (34); thus myocardial stunning must be viewed as a global derangement of the mechanical properties of the heart.

B. Myocardial Stunning After Multiple, Completely Reversible Ischemic Episodes

Repeated brief (2–10 min) coronary occlusions depress systolic function and result in prolonged contractile impairment despite absence of irreversible damage (10, 28a, 35, 117, 119, 133, 141, 142, 146, 147). This model of myocardial stunning differs from the single 10- or 15-min occlusion model in several respects: the mechanical dysfunction develops more gradually and is associated with a considerably greater total ischemic burden (20–60 min vs. 10–15 min), and its severity is not related to collateral perfusion during ischemia (28a, 35). Whether recurrent ischemic episodes have a preconditioning effect or a cumulative effect on contractile function in this model is not entirely clear. In dogs subjected to ten 5-min coronary occlusions, the first occlusion preconditions the myocardium against the next two occlusions so that the overall severity of stunning is the same after one or three occlusions. However, after the third occlusion, this preconditioning effect is negated, and additional occlusions cause a cumulative depression of contractility (28a). A similar paradigm (i.e., a partial preconditioning effect of the initial occlusions against subsequent occlusions) has been observed in conscious rabbits (155).

C. Myocardial Stunning After a Single, Partly Irreversible Ischemic Episode (Subendocardial Infarction)

In the dog, when reperfusion is instituted after a period of coronary occlusion >20 min but <3 h, the subendocardial portion of the region at risk is generally found to be infarcted, whereas variable quantities of subepicardial tissue remain viable (70). This subepicardial tissue salvaged by reperfusion may require days or weeks to recover its contractile function (30, 42, 90, 156). Thus early reperfusion during acute myocardial infarction results in an admixture of infarcted subendocardium and stunned subepicardium (i.e., irreversible and reversible dysfunction, respectively). Despite its important clinical

implications, myocardial stunning after subendocardial infarction has not been studied extensively because of numerous methodological problems. In particular, it is 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.

D. Myocardial Stunning After Global Ischemia In Vitro

Cellular viability in these preparations depends on many factors, including species, temperature, duration of ischemia, and perfusate composition. Although in these models the reversibility of the contractile abnormalities cannot be verified, under selected conditions isolated hearts reperfused after transient ischemia exhibit complete normalization of phosphocreatine content and intracellular pH (1, 2, 78, 86, 88, 98, 128, 143, 151), suggesting that viability is generally preserved. Accordingly, despite the numerous obvious differences from ischemia *in vivo*, myocardial stunning can be mimicked in isolated heart preparations. Obviously, the relevance to stunning becomes questionable in cases where these preparations are associated with significant cell death (3, 4, 32, 37, 51, 83, 108, 113, 138, 139, 167).

E. 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 (50, 67, 74, 144). The reversibility of these derangements has not been documented, but under carefully controlled conditions, they are likely to be due mostly to stunning.

F. Myocardial Stunning After Exercise-Induced Ischemia

Exercise-induced increases in myocardial oxygen demands in the face of limited supply (flow-limiting stenosis) may provoke myocardial ischemia and dysfunction in animals. These contractile abnormalities persist after cessation of exercise even if the stenosis is released (65, 66). Importantly, Vatner and colleagues (62) have shown that, in dogs with left ventricular hypertrophy, exercise can induce both ischemic myocardial dysfunction and post-

ischemic myocardial stunning in the absence of any coronary stenosis, an observation that could have major clinical implications. In summary, myocardial stunning can also occur after high-flow ischemia in which the primary problem is an increase in oxygen demand 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 (12). An important, unresolved issue is whether or not all forms of stunning share a common pathogenesis. To avoid potentially misleading generalizations, it seems prudent to recognize that concepts derived from one experimental setting cannot be automatically extrapolated to another one.

V. FACTORS THAT DETERMINE THE SEVERITY OF MYOCARDIAL STUNNING

As a general concept, the stunned myocardium is a "hypersensitive" myocardium (18); that is, factors that affect contractile performance in the normal, healthy myocardium have a greater impact on the stunned, convalescent myocardium. The factors that determine the severity of stunning after regional ischemia have been reviewed elsewhere (18) and include, among others, the severity and duration of flow deprivation, the myocardial temperature, the size of the ischemic region, and the loading conditions of the heart. The severity and duration of flow deprivation and the myocardial temperature are probably the most important. In conscious dogs undergoing a 15-min coronary occlusion, there is a close coupling between the degree of myocardial dysfunction after reperfusion and the collateral blood flow during the preceding period of ischemia, whereby even small differences in ischemic perfusion are associated with large differences in postischemic recovery (28). Furthermore, as discussed above, 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 (23). Another important factor is the duration of flow deprivation; the longer the ischemic period, the greater the ensuing mechanical abnormalities (123). Temperature is an enormously important but frequently overlooked determinant of stunning (18, 158); even small changes in myocardial temperature are associated with major changes in the severity of the contractile abnormalities (158).

The notion that the severity of postischemic dysfunction is determined to a large extent by the severity and duration of the antecedent ischemia has two important implications (12). First, whatever the precise mechanism responsible for stunning may be, such a mechanism must be initiated and modulated by perturbations associated with ischemia. Although stunning appears to be, in part, a

TABLE 2. *Mechanisms proposed for myocardial stunning*

<i>Most plausible</i>
Oxyradical hypothesis (generation of oxygen-derived free radicals)
Calcium hypothesis
Calcium overload
Decreased responsiveness of myofilaments to calcium
<i>Less plausible</i>
Excitation-contraction uncoupling due to sarcoplasmic reticulum dysfunction
<i>Not plausible</i>
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
Impaired excitation

form of reperfusion injury (see sect. x), it is ischemia that "primes" the myocardium for the development of such injury. Second, manipulations that attenuate the severity of ischemia would be expected to attenuate stunning after reflow. Indeed, reducing the severity of ischemia is probably the most effective way of reducing the severity of postischemic dysfunction (12), although interventions implemented at the moment of reflow can also be effective (see sects. VIII B and x).

VI. MECHANISM OF MYOCARDIAL STUNNING

Thus, in very general terms, postischemic dysfunction is modulated by abnormalities occurring during ischemia, but what is the specific sequence of events whereby transient ischemia leads to prolonged depression of contractility after flow is restored?

A number of hypotheses were proposed in the 1980s, most of which have been subsequently abandoned (Table 2) (these hypotheses are reviewed in Ref. 12). At present, the two viable theories regarding the pathogenesis of myocardial stunning are the oxyradical hypothesis and the calcium hypothesis (Table 2). As pointed out previously (12, 87), these theories are not mutually exclusive and probably represent different facets of the same pathophysiological process.

VII. THE OXYRADICAL HYPOTHESIS

A. Role of Oxyradicals in Stunning After a Single, Completely Reversible Ischemic Episode

Because most of the evidence supporting the oxyradical hypothesis has been obtained in models of

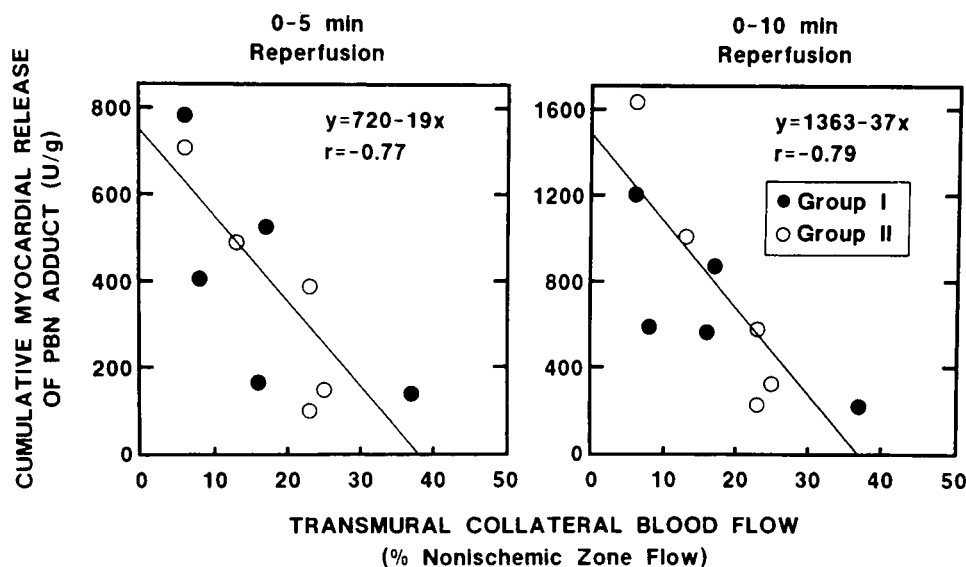


FIG. 1. Relationship between mean transmurial collateral blood flow to ischemic region during coronary occlusion (horizontal axis) and total cumulative myocardial release of α -phenyl-*N*-*tert*-butylnitron (PBN) adducts during first 5 min (left) and 10 min (right) of reperfusion in open-chest dogs. Myocardial stunning was produced by a 15-min coronary artery occlusion followed by reperfusion. The spin trap PBN was infused directly into the occluded/reperfused coronary artery to measure myocardial release of free radicals (which is reflected in the level of PBN adducts in coronary venous blood returning from ischemic-reperfused region). Collateral flow is expressed as percentage of simultaneous nonischemic zone flow; adduct release is expressed in arbitrary units per gram of myocardium. Group I, PBN given before ischemia; group II, PBN given at reperfusion. In both groups, myocardial production of PBN adducts after coronary reperfusion was linearly and inversely related to collateral flow during antecedent occlusion. These data suggest that the severity of ischemic injury is major determinant of severity of subsequent reperfusion injury. [From Bolli et al. (24), *The Journal of Clinical Investigation*, 1988, by copyright permission of the American Society for Clinical Investigation.]

myocardial stunning produced by a single, completely reversible episode of regional ischemia (i.e., a 15-min coronary occlusion in a dog), it is appropriate to devote the larger part of this section to a discussion of the role of oxygen radicals in these models.

1. Effect of antioxidants on myocardial stunning

In the early 1980s, a number of investigators postulated that myocardial stunning is caused in part by the generation of reactive oxygen species [e.g., superoxide anion ($\cdot\text{O}_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot\text{OH}$)]. This hypothesis was tested in a series of studies conducted in open-chest dog preparations in which the left anterior descending coronary artery was occluded for 15 min and then reperfused. (The advantage of this model, as indicated above, is that the mechanical derangements observed after reperfusion can be entirely ascribed to stunning.) In the first experiment to test this idea (115), administration of superoxide dismutase (SOD) (which catalyzes the dismutation of $\cdot\text{O}_2^-$ to O_2 and H_2O_2) and catalase (which reduces H_2O_2 to O_2 and H_2O) significantly enhanced recovery of function after reperfusion. Similar findings with SOD and catalase were subsequently reported by other investigators using similar models (53, 82, 112, 124). Dimethylthiourea and mercaptopropionyl glycine (MPG), two scavengers of $\cdot\text{OH}$, were also found to

produce a significant and sustained improvement in the function of the stunned myocardium (20, 27, 114), suggesting that the $\cdot\text{OH}$ is an important mediator of post-ischemic dysfunction. In addition, the iron chelator desferrioxamine was found to attenuate postischemic dysfunction (26, 43), presumably through prevention of the iron-catalyzed formation of $\cdot\text{OH}$ (through the Haber-Weiss or Fenton mechanisms). In the ensuing years, numerous other studies have demonstrated the ability of a wide variety of antioxidants, targeted at different steps of the univalent pathway of reduction of oxygen, to attenuate myocardial stunning after a 15-min coronary occlusion in different animal species, including rabbits and pigs (reviewed in Ref. 17).

2. Direct evidence for the oxyradical hypothesis

Despite this impressive body of evidence supporting the oxyradical hypothesis, all of these studies (20, 26, 27, 43, 53, 73, 82, 112, 114, 115, 124) were limited by the fact that the evidence for a causative role of oxygen metabolites in postischemic dysfunction was indirect. Therefore, to validate the oxyradical hypothesis of stunning, it was necessary to directly demonstrate and quantitate free radical generation in the stunned myocardium in the presence and absence of antioxidant interventions.

Accordingly, the spin trap α -phenyl-*N*-*tert*-butylini-

trone (PBN) and electron paramagnetic resonance (EPR) spectroscopy were used to detect and measure production of free radicals in a series of studies conducted in the same open-chest dog model of postischemic dysfunction (15-min coronary in vivo occlusion) in which antioxidants had proven protective (see above). In the initial study (24), a burst of free radical production was demonstrated immediately after reperfusion. Furthermore, a linear, positive relation was noted between the magnitude of free radical production and the magnitude of ischemic flow reduction (Fig. 1), indicating that the intensity of free radical generation after reflow is proportional to the severity of the antecedent ischemia (24); hence, 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 alleviate the severity of ischemia will indirectly attenuate free radical reactions after reflow (see sect. x). Subsequent studies demonstrated that SOD plus catalase (21), MPG (Fig. 2) (20), and desferrioxamine (25) suppressed the production of free radicals in the stunned myocardium and, at the same time, attenuated postischemic dysfunction, suggesting a cause-and-effect relationship between the production of free radicals in the stunned myocardium and the depression of contractility.

Although PBN is highly sensitive in detecting oxyradicals, it does not provide specific information regarding the chemical nature of the species generated. Accordingly, a different technique (aromatic hydroxylation of phenylalanine) was used to specifically interrogate the role of $\cdot\text{OH}$ in myocardial stunning in open-chest dogs (145). Generation of hydroxylated derivatives of phenyl-

alanine (*ortho*-, *meta*-, and *para*-tyrosine) was observed during the first few minutes of reperfusion after a 15-min occlusion, indicating that $\cdot\text{OH}$ is produced in the stunned myocardium upon reperfusion (Fig. 3A); moreover, $\cdot\text{OH}$ scavengers suppressed tyrosine production and attenuated the dysfunction (Fig. 3B), suggesting a key role of $\cdot\text{OH}$ as a mediator of stunning (145). The similarity of the results obtained with two completely different techniques (spin trapping, Refs. 20–22, 24, 25; and aromatic hydroxylation, Ref. 145) further corroborates the concept that reactive oxygen species play a significant role in the pathogenesis of postischemic ventricular dysfunction. Generation of free radicals has also been demonstrated by a number of studies in in vitro models of myocardial ischemia-reperfusion associated with infarction (e.g., Ref. 168), indicating that the release of global ischemia is also associated with formation of free radical species. More recently, studies have demonstrated that free radicals are generated after 20 min of global ischemia in isolated rat hearts (a model likely to be associated mostly with stunning, with no or little infarction) and that antioxidant agents attenuate free radical formation and, at the same time, alleviate both postischemic dysfunction, again supporting a causative role of free radicals in myocardial stunning after global ischemia (136).

3. Role of oxyradicals in conscious animals

Although the studies discussed above (20–22, 24–27, 43, 53, 73, 82, 112, 114, 115, 124, 145) consistently supported the oxyradical hypothesis, their significance was limited by the fact that they had been performed in open-

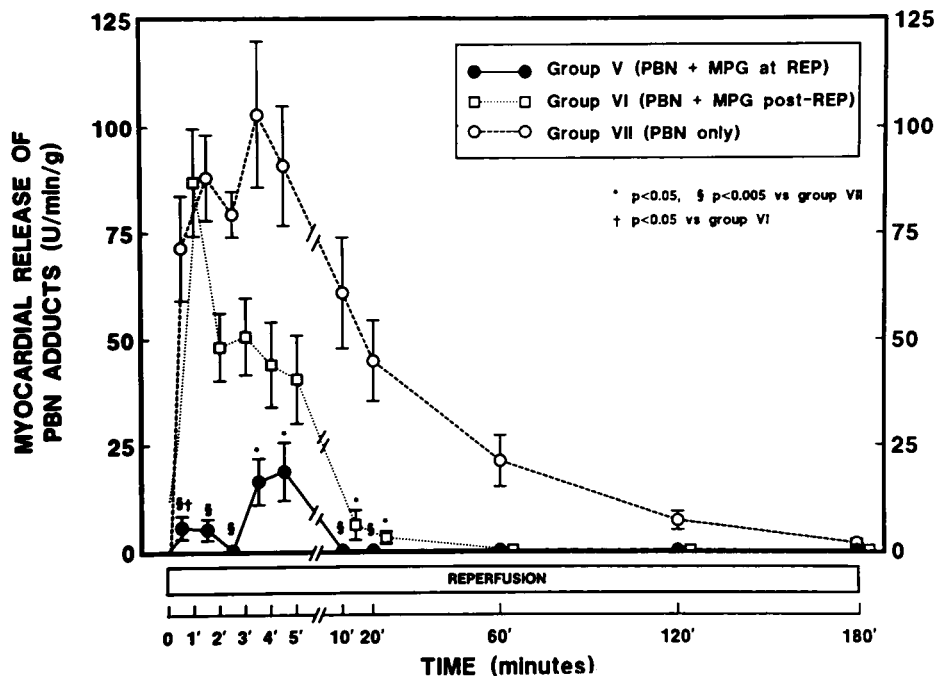


FIG. 2. Time course of myocardial release of PBN adducts in group V [mercaptopyropionyl glycine (MPG) infusion started 1 min after reperfusion; $n = 5$], group VI (MPG infusion started 1 min after reperfusion; $n = 5$), and group VII (controls; $n = 6$). Myocardial stunning was produced in open-chest dogs by a 15-min coronary occlusion followed by reperfusion. The antioxidant MPG was infused directly into the occluded-reperfused coronary artery. Myocardial production of free radicals was assessed using the spin trap PBN, which reacts with free radicals to form PBN adducts. PBN was given by intracoronary route. Infusion of MPG started 1 min before reperfusion markedly suppressed production of free radicals in stunned myocardium. Infusion of MPG started 1 min after reperfusion did not affect initial production of free radicals and produced a delayed suppression that became evident by 10 min of reflow. Because only MPG given as in group V attenuated postischemic dysfunction (whereas MPG given as in group VI did not) (see Fig. 11), these data suggest that the free radicals important in myocardial stunning are those generated immediately after reperfusion. Data are means \pm SE. [From Bolli et al. (20), with permission. Copyright 1989 American Heart Association.]

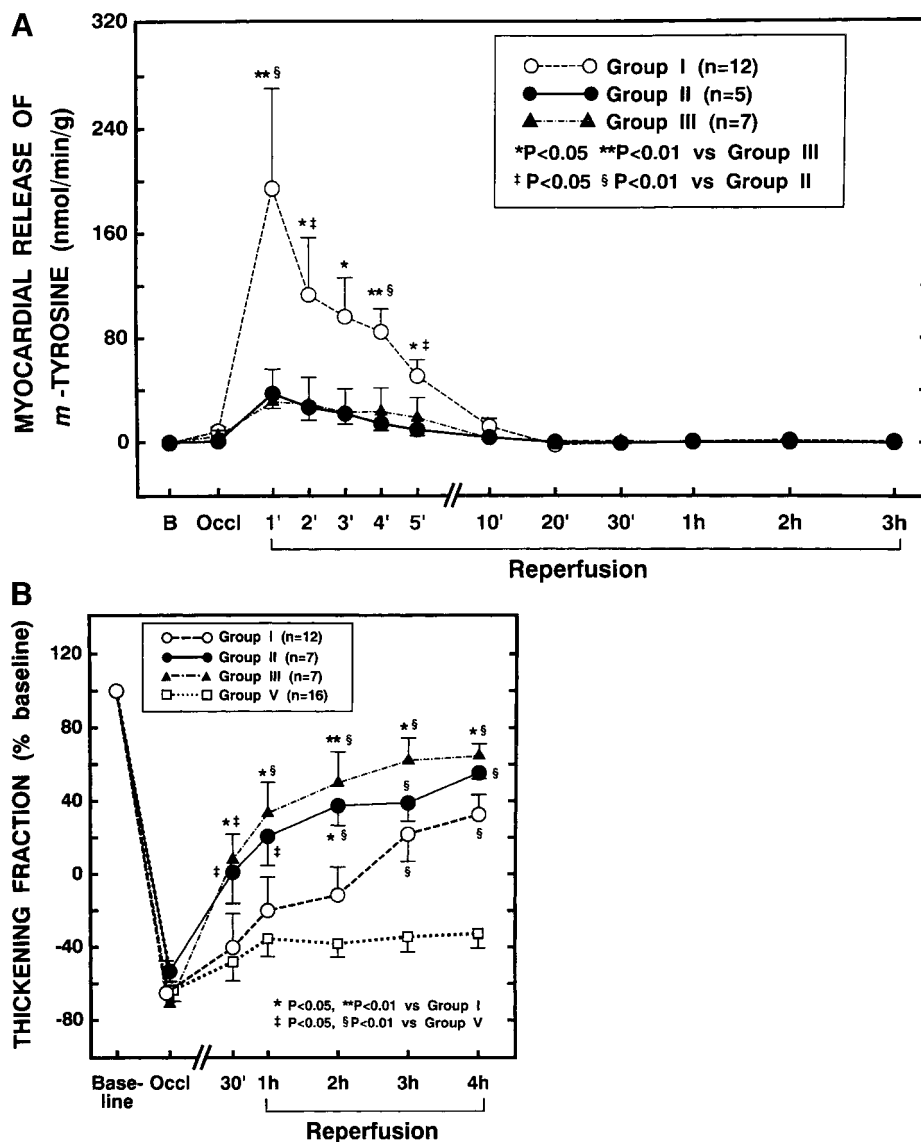


FIG. 3. A: time course of myocardial release of *m*-tyrosine in *group I* (phenylalanine only, $n = 12$), *group II* (phenylalanine + MPG, $n = 5$), and *group III* (phenylalanine + MPG, superoxide dismutase, catalase, and desferrioxamine, $n = 7$). Open-chest dogs were subjected to a 15-min coronary occlusion followed by reperfusion and were given phenylalanine only or phenylalanine in combination with various antioxidants. Phenylalanine reacts with hydroxyl radical to form hydroxylated derivatives, including *m*-tyrosine. Therefore, release of *m*-tyrosine in the coronary venous blood after reperfusion is an index of hydroxyl radical formation in the ischemic-reperfused myocardium. In *group I*, reperfusion after a 15-min left anterior descending coronary artery occlusion was associated with a burst of *m*-tyrosine production. Release of *m*-tyrosine peaked 1 min after reperfusion and then declined substantially by 10 min. Production of *m*-tyrosine was markedly decreased in *groups II* and *III*. B, baseline; Occl, coronary occlusion. Data are means \pm SE. *B*: systolic thickening fraction in the ischemic-reperfused region 5 min after coronary occlusion (Occl) and at selected times after reperfusion in the following groups: *group I* (phenylalanine only, $n = 12$), *group II* (phenylalanine + MPG, $n = 7$), *group III* (phenylalanine + MPG, superoxide dismutase, catalase, and desferrioxamine, $n = 7$), and *group V* [controls (no phenylalanine), $n = 16$]. Control dogs (*group V*) exhibited little recovery of contractile function after reperfusion. In *group I*, which received phenylalanine only, recovery of wall thickening was greater than in controls, indicating that phenylalanine, in itself, attenuated myocardial stunning. In *group II*, which received phenylalanine + MPG, recovery of wall thickening was further enhanced compared with *group I*; in *group III*, which received phenylalanine + MPG, superoxide dismutase, catalase, and desferrioxamine, there was a slight additional improvement in recovery of function, but differences versus *group II* were not statistically significant. Notice that a "broad spectrum" antioxidant therapy failed to completely prevent myocardial stunning, despite the fact that this same treatment almost completely abolished hydroxyl radical production following reperfusion in stunned myocardium, as shown in A. This suggests that oxyradicals are not the sole cause of this abnormality. Thickening fraction is expressed as percent of baseline values. Data are means \pm SE. [From Sun et al. (145), with permission. Copyright 1993 American Heart Association.]

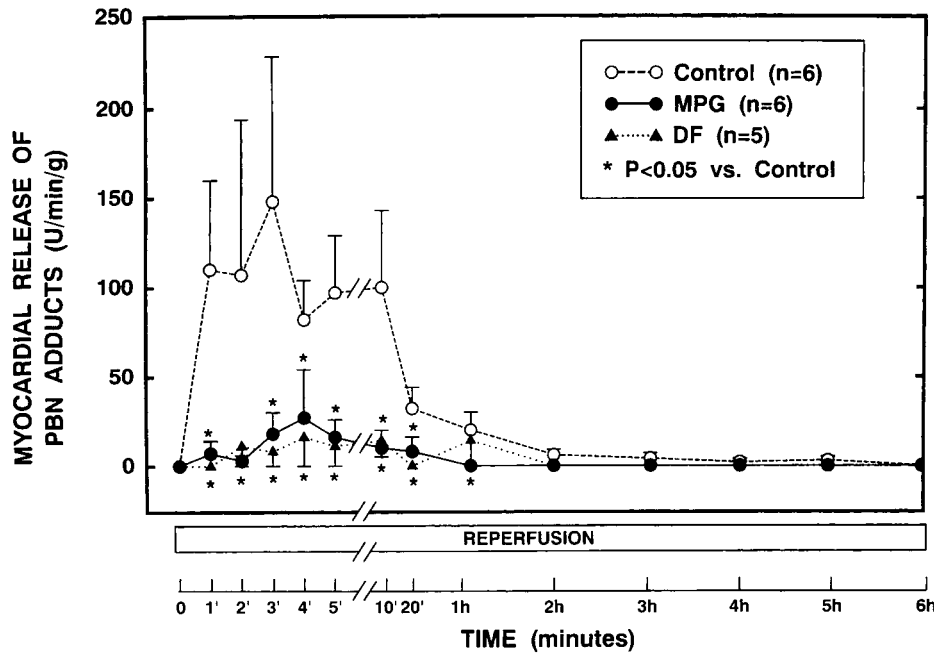


FIG. 4. Myocardial release of PBN adducts in control dogs, MPG-treated dogs, and desferrioxamine (DF)-treated dogs. Conscious dogs undergoing a 15-min coronary occlusion received an intravenous infusion of PBN 5 min before occlusion and ending 10 min after reperfusion. Dogs were either not treated with antioxidants (controls) or received an infusion of MPG or DF. Notice that both MPG and DF suppressed the burst of free radical generation that occurred in controls after reperfusion. Data are means \pm SE. [From Sekili et al. (135), with permission. Copyright 1993 American Heart Association.]

chest animals. Thus artifacts due to the combined effects of anesthesia, hypothermia, surgical trauma, volume and ionic imbalances, unphysiological conditions, cytokine release, and attending neurohumoral perturbations, as well as other potentially confounding variables, could not be excluded. This concern became even more compelling when it was shown that both the severity of myocardial stunning (158) and the magnitude of free radical generation (91) after a 15-min coronary occlusion are greatly exaggerated in open-chest dogs as compared with conscious dogs, even when differences in collateral flow are taken into account and fundamental physiological variables in the open-chest preparation are carefully kept within normal limits. These striking differences between

the two models indicated the presence of artifacts in the open-chest dog model and raised the possibility that results obtained in this model may not be applicable to more physiological conditions. It was therefore important that the oxyradical hypothesis be verified in conscious animal preparations. Studies in conscious dogs subjected to a 15-min coronary occlusion demonstrated, using EPR spectroscopy, 1) that free radicals are generated following reperfusion, with a burst peaking at 2–3 min after reflow and abating within 20 min (Fig. 4) (91, 135); 2) that antioxidants (desferrioxamine and MPG) markedly attenuate this burst of free radical generation (Fig. 4) (135); and 3) that these same antioxidants also attenuate myocardial stunning (Fig. 5) (135, 158), indicating that free

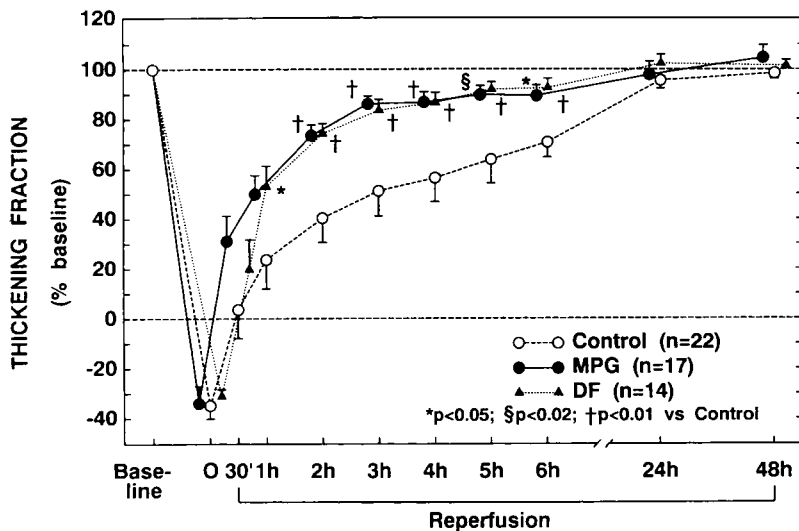


FIG. 5. Systolic thickening fraction in the ischemic-reperfused region 5 min after coronary occlusion (O) and at selected times after reperfusion. Conscious dogs underwent a 15-min coronary occlusion followed by reperfusion and received no antioxidant treatment (controls) or MPG (a hydroxyl radical and peroxynitrite scavenger) or DF (an iron chelator). Notice that both MPG and DF markedly enhanced recovery of regional myocardial function (measured as a systolic thickening fraction) throughout reperfusion phase. Thickening fraction is expressed as a percentage of baseline values. Data are means \pm SE. [From Sekili et al. (135), with permission. Copyright 1993 American Heart Association.]

radicals are necessary for myocardial stunning to occur. Taken together, these results (91, 135, 158) indicate that the oxyradical hypothesis of myocardial stunning is applicable to the conscious animal preparation, i.e., to the most physiological animal preparation available.

In summary, numerous investigations from several independent laboratories and in a variety of models (20–22, 24–27, 43, 53, 73, 82, 91, 112, 114, 115, 124, 135, 145, 158) uniformly suggest that reactive oxygen species play a significant role in the genesis of myocardial stunning after a 15-min period of ischemia, both in open-chest and in conscious animals. At the time of this writing, there are at least 22 full-length published articles examining the effect of antioxidants on myocardial stunning after a brief (15-min) coronary occlusion; all of these studies [except those that used superoxide dismutase alone (73) or catalase alone (73)] have uniformly shown a protective effect of antioxidants against stunning (reviewed in Ref. 17). This is indeed a rare example of concordance among different investigators, particularly in the area of free radical-mediated injury. This concordance is in striking contrast to the controversy that surrounds the role of oxyradicals in myocardial infarction (13).

4. Mechanism of oxyradical-mediated contractile dysfunction

Oxygen metabolites have been shown to depress myocardial function both in vitro and in vivo (reviewed in Ref. 12). The exact 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 can attack non-specifically virtually all cellular components. Theoretically, every abnormality described thus far in the stunned myocardium could be caused by oxyradicals. At least two key cellular components, proteins and lipids, could be the targets of free radical-initiated reactions, leading to protein denaturation and enzyme inactivation as well as peroxidation of the polyunsaturated fatty acids contained in cellular membranes (12, 57). The latter effect would impair selective membrane permeability and interfere with the function of various cellular organelles (12).

The sarcolemma may be a critical target of free radical-mediated damage (Fig. 6), since oxyradicals interfere with its calcium transport and calcium-stimulated ATPase activity (75, 76). Oxygen radicals have also been shown to interfere with Na^+ - Ca^{2+} exchange (57, 130) and to inhibit Na^+ - K^+ -ATPase activity (57, 77). Impairment of Na^+ - K^+ -ATPase activity results in sodium overload, with consequent activation of the Na^+ - Ca^{2+} exchange activity (52, 57, 131). These observations imply that excessive production of oxyradicals

could result in increased transsarcolemmal calcium influx and cellular calcium overload (Fig. 6). It is also plausible that oxyradicals cause decreased responsiveness of myofilaments to calcium by producing selective damage of contractile proteins, for example, by oxidation of critical thiol groups (150) (Fig. 6). In this regard, exposure of myofilaments to superoxide anion has been shown to result in a dose-dependent reduction in maximal calcium-activated force (with no alteration in calcium sensitivity) (92). Interestingly, myofilaments were found to be very sensitive to superoxide, with significant force reduction being observed after exposure of <1 min to the superoxide-generating system, suggesting that cross-bridge kinetics are highly vulnerable to superoxide anion (92). Finally, oxyradicals have been shown to impair sarcoplasmic reticulum function (57, 132). It is important to point out that the foregoing postulated mechanisms involve alterations in calcium homeostasis and thus would help to reconcile the oxyradical hypothesis and the calcium overload hypothesis of stunning into one pathogenetic mechanism (Fig. 6).

5. Sources of oxyradicals in the stunned myocardium

The exact sources of oxyradical production in the stunned myocardium remain unclear. In the canine and rat models of myocardial stunning, xanthine oxidase appears to be a source of free radicals (33, 164), whereas it is now definitely established that neutrophils are unimportant (16, 118). The role of xanthine oxidase in humans is uncertain because data regarding the myocardial content of this enzyme in the human heart are conflicting (40, 69). The presence of xanthine oxidase, however, does not appear to be necessary for the oxyradicals that cause stunning to be formed, since antioxidants attenuate stunning in species [pigs (147) and rabbits (82, 153)] in which the myocardium contains little or no xanthine oxidase activity. Interpretation of the effects of xanthine oxidase inhibitors is complicated by the fact that these agents have major, unanticipated effects to increase the responsiveness of the myofilaments to calcium (121). There are several other processes that could generate free radicals during reperfusion, including activation of the arachidonate cascade, autoxidation of catecholamines and other compounds, activation of various NAD(P)H oxidases, and, perhaps more importantly, damage of the mitochondrial electron transport chain.

B. Role of Oxyradicals in Other Forms of Myocardial Stunning

The investigations reviewed thus far employed a single brief (15 min) coronary occlusion. Do oxyradicals also

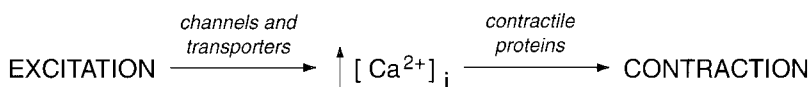


FIG. 7. Schematic representation of the excitation-contraction coupling process. $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration.

sults suggest that short-term administration of antioxidant enzymes is not effective in mitigating myocardial stunning associated with subendocardial infarction, perhaps because the pathogenesis of postischemic dysfunction is different when this abnormality is caused by a prolonged period of ischemia. However, other studies (45, 80, 127) have shown that the cell-permeant antioxidants oxypurinol, *N*-acetylcysteine, and Trolox attenuate myocardial stunning independently of infarct size limitation in closed-chest dogs subjected to 90 min of coronary occlusion and 24 h of reflow (45, 127) and in open-chest pigs subjected to 45 min of coronary occlusion and 72 h of reperfusion (80). Exercise-induced stunning is not alleviated by SOD and catalase (64).

In summary, there is strong evidence that oxyradicals contribute to postischemic dysfunction after global ischemia (in vitro as well as in vivo) and after multiple episodes of regional ischemia (Table 1). There is presently no evidence that they contribute to exercise-induced postischemic dysfunction. The role of oxygen radicals in myocardial stunning after a prolonged, partly irreversible ischemic insult remains uncertain and represents a major unresolved problem (Table 1). 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.

VIII. THE CALCIUM HYPOTHESIS

In a very broad sense, the calcium hypothesis postulates that stunning is the result of a disturbance of cellular calcium homeostasis. This hypothesis encompasses three distinct postulated mechanisms: decreased responsiveness of the contractile protein machinery to calcium, calcium overload, and excitation-contraction uncoupling due to sarcoplasmic reticulum dysfunction (Table 2). Given its multiple biological functions, it is natural that cellular calcium homeostasis has come under particularly close scrutiny. Calcium plays two distinct roles in myocardial stunning: 1) as the chemical activator of contraction, calcium is a major determinant in the pathophysiology of the dysfunction; and 2) as an agent of injury, calcium may contribute to the pathogenesis of stunning.

A. Role of Calcium in the Pathophysiology of Myocardial Stunning

1. Overview of cardiac excitation-contraction coupling

Changes in cardiac contractile force can be effected at each of three steps: 1) modulation of intracellular free

calcium concentration ($[Ca^{2+}]_i$), 2) modulation of the contractile protein response to $[Ca^{2+}]_i$, and 3) loading. Given that the characterization of excitation-contraction coupling has been performed primarily under isometric conditions in isolated preparations, only *factors 1* and *2* are considered here. Nevertheless, loading, particularly the left ventricular chamber pressure generated by non-ischemic areas of the heart, undoubtedly shapes the stunning response in a major way in in vivo models of regional ischemia.

Each of the steps in excitation-contraction coupling can be broken down into various components (11). Figure 7 lumps the various processes into two steps. A lesion between excitation and calcium availability would motivate extensive examination of the various pathways that mediate calcium influx and efflux from the cytosol, particularly ion channels and transporters. If calcium cycling is shown not to be the limiting factor, attention logically turns to the next step, the response of the contractile proteins to calcium. This step consists of three components: the maximal force-generating capacity of the myofilaments (at saturating levels of $[Ca^{2+}]_i$), the sensitivity of the myofilaments to calcium (i.e., the range of $[Ca^{2+}]_i$ which activates the contractile proteins), and cross-bridge cycling kinetics. When speaking generally of the ability of the contractile machinery to generate force in response to calcium, it is appropriate to use the term *myofilament responsiveness*, which encompasses all three of the aforementioned mechanisms.

2. Excitation-contraction coupling in stunned myocardium

The site of the lesion in excitation-contraction coupling has recently been the focus of intensive investigation. Because electrical activation is normal (55), the basis for stunned myocardium must lie in either of two broad mechanistic categories (Fig. 7). First, the availability of activator calcium might be restricted; such an effect could be mediated by abnormal calcium entry into (or removal from) the cytosol due to lesions in one or more cellular calcium-handling pathways. Alternatively, the responsiveness of the contractile machinery to calcium might be blunted such that the myocardium generates less force for any given rise of $[Ca^{2+}]_i$ (i.e., the calcium transient); in this case, the availability of calcium need not be the limiting factor.

A) STUDIES IN ISOLATED HEARTS. The last 12 years of research have overwhelmingly implicated the myofilaments as the site of the critical lesion in stunning, at least in isolated perfused heart models. The first clue that

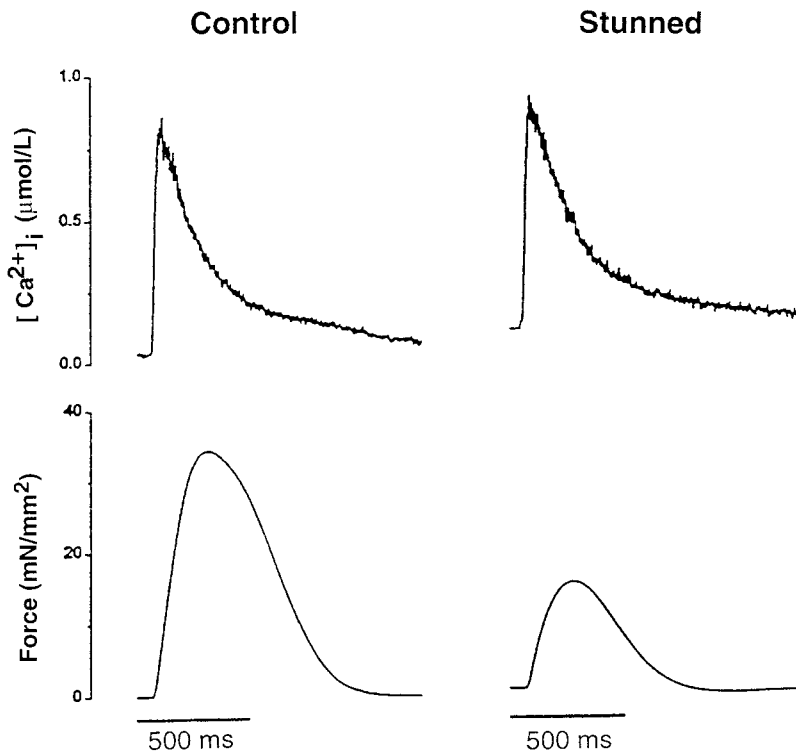


FIG. 8. Measurements of activator calcium (*top*) and contractile force (*bottom*) in representative control and stunned muscles reveal uncoupling of calcium and contraction. Note that calcium availability is not deficient; the problem lies downstream, at the level of the contractile machinery. Records are from trabeculae dissected from rat hearts that had been perfused nonischemically for 1 h (*left*) or that had been subjected to 20-min global ischemia followed by reperfusion (*right*) at 37°C. Muscles were then isolated, mounted isometrically, and microinjected with the calcium indicator fura 2. Calcium transients and force were recorded in 1 mM external calcium at 22°C. [From Gao et al. (46), with permission. Copyright 1995 American Heart Association.]

myofilament function was abnormal came from Kusuoka et al. (88), who found a depressed maximal calcium-activated pressure (the whole heart equivalent of maximal calcium-activated force) in stunned ferret hearts. Marbán and co-workers (95, 96) later developed and validated the methodology for measuring $[Ca^{2+}]_i$ in isolated perfused ferret hearts by NMR spectroscopy. Calcium transients turned out to be comparable before and after ischemia; if anything, there was a tendency for systolic $[Ca^{2+}]_i$ to increase in the stunned hearts despite a 40% drop in developed ventricular pressure (86). Carrozza et al. (31) later confirmed that calcium transients are not depressed in stunned ferret hearts using a complementary calcium-measurement method, aequorin loaded into the epicardial interstitium.

Gao et al. (46) examined excitation-contraction coupling in stunned myocardium more directly using experimental preparations devoid of complications from the superimposed effects of vascular turgor and loading. Measurements of $[Ca^{2+}]_i$ and force in thin ventricular trabeculae from nonischemic and stunned rat hearts confirmed that calcium transients are not reduced in stunned myocardium (Fig. 8). Functional studies of the myofilaments during steady-state calcium activation revealed both a decrease in maximal force and a decrease in sensitivity (i.e., a rightward shift of the $[Ca^{2+}]_i$ -force relationship) (Fig. 9). This study further showed that simple changes of cross-bridge attachment and detachment rates in a quantitative model of myofilament interaction reproduced the

salient features of the contractile dysfunction of stunned myocardium.

Whether the decreased calcium responsiveness of the myofilaments is due to decreased maximal calcium-activated force, decreased calcium sensitivity, or both, remains controversial. Kusuoka and co-workers (86, 88) argued that both of these fundamental aspects of myofilament function are depressed in postischemic hearts. Gao et al. (46) confirmed this conclusion while emphasizing that, quantitatively, the decrease of maximal force is the single most important factor. Carrozza et al. (31), on the other hand, concluded that only maximal calcium-activated force is depressed. One limitation with regard to the interpretation of their data was uncertainty regarding the maximal level of activation, because saturation of force with respect to $[Ca^{2+}]_i$ was not clearly achieved.

B) STUDIES IN SKINNED PREPARATIONS. Although much of the evidence supporting a lesion at the level of the myofilaments comes from intact myocardium, supporting evidence has also been obtained in chemically skinned preparations. Hofmann et al. (63) measured myofilament calcium sensitivity in a porcine *in vivo* model of stunning produced by 45 min of hypoperfusion followed by reflow. They found that calcium sensitivity is indeed decreased in postischemic skinned heart cells, without a resolvable change in maximal force (possibly reflecting the large variation in the absolute values of maximal force, which ranged from ~55 to ~150% of the mean value). These investigators subsequently reported that the decrease in

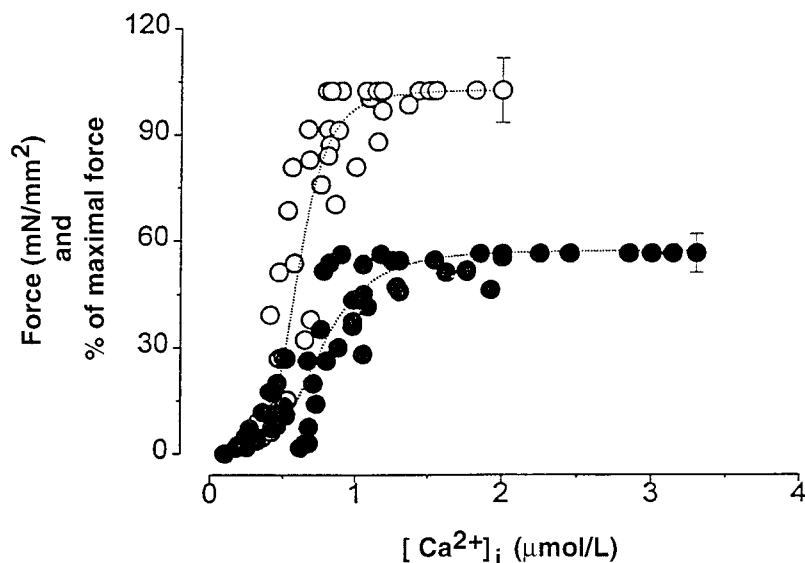


FIG. 9. Steady-state contractile activation in intact muscles reveals marked depression of maximal force and a modest but significant desensitizing shift (the midpoint of calcium activation increased from 0.60 to 0.82 μM). [From Gao et al. (46), with permission. Copyright 1995 American Heart Association.]

calcium sensitivity occurs only upon reperfusion (109), confirming a key prediction of the reflow/proteolysis hypothesis outlined below. Investigators from the same laboratory (103) also found that some “stunned” cells do not exhibit alterations in steady-state calcium activation; this is the routine method for assessing myofilament sensitivity, in which calcium is kept elevated for long periods to allow the force response to reach a steady level. Nevertheless, such cells did exhibit clear-cut changes in the kinetics of cross-bridge cycling (as manifested by marked slowing of force redevelopment after quick releases under conditions of maximal calcium activation). Such kinetic changes can effectively uncouple $[\text{Ca}^{2+}]_i$ from force, as shown experimentally and theoretically with the negatively inotropic drug butanedione monoxime (7). Dietrich et al. (37) found no changes in steady-state myofilament calcium responsiveness following reflow after long periods of total ischemia (40 min) in isolated rat hearts; cross-bridge kinetics were not examined. Although the authors referred to this as “stunned myocardium,” 40 min of total ischemia is known to produce significant irreversible injury in addition to stunning (see sect. IV D). More recently, Van Eyk et al. (160) examined the relationship between duration of ischemia and reflow on myofilament properties in skinned fiber bundles from isolated rat hearts. They found a marked depression of maximal force ($\sim 45\%$) when ≥ 15 min of ischemia was followed by reflow, but no depression with 15 min of ischemia alone. Interestingly, the functional lesion of the myofilaments in that study was entirely attributable to a reduction of maximal force; sensitivity was actually slightly greater (i.e., shifted to lower $[\text{Ca}^{2+}]_i$) in both the ischemic and ischemic/reperfused groups compared with nonischemic controls.

Although support for dysfunction of the contractile

proteins is strong in studies with isolated muscle specimens, there is little evidence for or against this idea in vivo. Ito et al. (68) have argued, based on regional calcium infusion in an in vivo canine model of stunning, that the ventricular response to calcium is not impaired. It is important to recognize, however, that absolute force generation was not quantified in that study (indeed, it cannot be easily quantified in vivo); it is possible that the relative response was preserved (as these authors showed), while the absolute magnitude might have been blunted. This is the conclusion that Heusch et al. (59) reached in their study of postischemic calcium responsiveness in a porcine in vivo model of myocardial stunning; maximal calcium responsiveness was decreased, but the relative responsiveness was preserved (when the responses were normalized relative to the greatest response in either control or stunned myocardium). The observation that stunned myocardium remains responsive to changes in extracellular calcium is entirely consistent with the idea that the principal lesion is at the maximal force-generating level. Relative recruitment of contractility by submaximal activations remains unimpaired, since the mechanisms of calcium cycling remain intact.

In summary, there is unanimous agreement that calcium availability is not limited in stunned myocardium. This is an important finding, because it implies that the mechanism of excitation-contraction uncoupling occurs distal to calcium availability, at the level of the contractile proteins (Fig. 7). Various studies have verified directly or indirectly myofilament dysfunction in intact or skinned samples of stunned myocardium; there is, however, some discrepancy in the literature as to the precise nature of this dysfunction. In intact muscle, the single greatest problem appears to be a reduction of maximal calcium-activated force in stunned

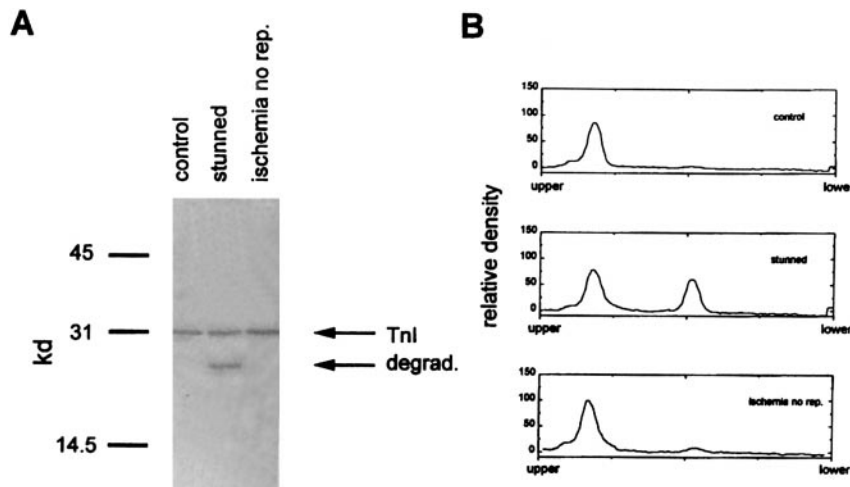


FIG. 10. Immunoblot analysis reveals degradation of the thin filament regulatory protein troponin I (TnI) in stunned myocardium. *A*: representative Western blot with lanes containing control muscle (*left*), stunned muscle after 20-min ischemia and reperfusion (*middle*), and a muscle from a heart that had been rendered ischemic for 20 min but not reperfused (*right*). *B*: densitometric analysis of blot in *A* reveals wild-type TnI in all lanes (~31 kDa molecular mass) and a degradation product of ~26 kDa only in stunned muscle. This degradation product has subsequently been shown, by Van Eyk and co-workers (103a), to correspond to a COOH-terminal truncation of 17 residues leaving behind amino acids 1–193 of TnI. [From Gao et al. (47), with permission. Copyright 1996 American Heart Association.]

myocardium, i.e., the greatest force that the muscle can possibly generate is markedly reduced. As discussed in section VIII A 3, this may be due to proteolytic injury to the contractile proteins and/or to oxyradical-mediated covalent modifications.

3. Mechanism of decreased calcium responsiveness

The mechanism underlying the decreased calcium responsiveness is not conclusively established at present, although the available evidence points to structural modifications of one or more myofibrillar (or myofibril-associated) proteins. The results from skinned myocardium, while somewhat disparate, suggest that stunning reflects alterations within the myofilaments themselves (49). This is an important point where the calcium and oxyradical hypotheses could potentially converge. As indicated above, there is abundant evidence implicating oxygen radicals in the pathogenesis of myocardial stunning. Among their multiple actions, oxyradicals could effect modifications of myofibrillar proteins (e.g., by oxidation of thiol groups; Ref. 150), resulting in impaired calcium responsiveness (92) (see sect. VII A 4). In addition to structural modifications of the myofilaments, other mechanisms may influence the calcium sensitivity of stunned myocardium *in vivo*. For example, in addition to their effects on calcium homeostasis and contractile proteins, oxygen radicals can cause a decrease in the content of reduced glutathione and an increase in oxidized glutathione (44). Oxidized glutathione has been shown to decrease calcium sensitivity in skinned cardiac muscles, whereas reduced glutathione has the opposite effect (9). Thus changes in the redox state of cytosolic glutathione may contribute to the desensitization of the stunned myofilaments. Nevertheless, examination of the same muscles before and after chemical membrane solubilization enabled Gao et al. (49) to demonstrate that the cardinal changes they had

observed in intact stunned myocardium (markedly decreased maximal force and modestly reduced myofilament sensitivity) persist after skinning. This observation points to the primacy of alterations within the proteinaceous matrix of the cell, as opposed to freely soluble factors [such as magnesium (110) or glutathione] which would have been equalized after skinning.

The findings that myofilament function is depressed, and that this depression persists after skinning, have motivated structural analysis of various key proteins within the contractile apparatus. Using immunohistochemistry, Matsumura et al. (101) observed patchy degradation of α -actinin, a myofilament-associated scaffolding protein, in globally stunned rat myocardium. Gao et al. (47) used immunoblots to analyze various key proteins involved in cross-bridge cycling. Among these, the thin filament regulatory protein troponin (Tn) I was unique in exhibiting partial degradation in stunned myocardium (but not in ischemic non-reperfused samples) (Fig. 10). The degradation of TnI could be prevented by modifications of the reperfusate designed to mitigate calcium overload and improve functional recovery. Van Eyk et al. (160) confirmed the observations of TnI and α -actinin degradation and identified a number of other alterations with prolonged ischemia and/or reflow (see also Refs. 8, 157, 163). The observation that TnI is partially degraded in stunned myocardium is particularly intriguing given the crucial role of this protein as an intermediary between calcium activation and cross-bridge cycling. It is possible, but as yet unproven, that the lesion of TnI suffices to explain the depressed myofilament function that underlies stunning. This idea becomes even more attractive when interpreted in light of evidence, summarized in section VIII B, supporting a role for calcium overload and calcium-activated proteolysis in the pathogenesis of stunning.

B. Role of Calcium in the Pathogenesis of Myocardial Stunning

1. Evidence for a role of calcium overload after reperfusion

The evidence reviewed above suggests that a decrease of myofilament calcium responsiveness features prominently in the pathophysiology of stunning, at least in *in vitro* models. Does calcium also play a role in the pathogenesis of stunning? Even in the mid 1980s, several lines of evidence already suggested that reperfusion injury, at least in its irreversible form, was associated with calcium overload. Grinwald (52) had elaborated a specific hypothesis to explain the mechanism of reperfusion-induced calcium overload, as follows: during ischemia, intracellular sodium ($[Na^+]_i$) accumulates due to energy depletion, but Na^+-Ca^{2+} exchange is inhibited by the concomitant acidosis. Upon reperfusion, the rapid reversal of acidosis reactivates Na^+-Ca^{2+} exchange at a time when sodium overload has not yet resolved, driving calcium into the cells (Fig. 6). Kusuoka et al. (88) tested this hypothesis explicitly in isolated ferret hearts and determined that reperfusion with low-calcium solutions markedly attenuates stunning, suggesting that calcium overload upon reflow, occurring by whatever mechanism, might be linked to stunning. Consistent with this notion, transient calcium overload, even in the absence of ischemia, leaves behind long-lasting functional sequelae identical to those of stunned myocardium (79). Grinwald's hypothesis (52) also predicted that persistence of acidosis during early reflow may be protective, by preventing the sudden reactivation of Na^+-Ca^{2+} exchange. Kitakaze et al. (78) tested this idea and found a striking prevention of stunning when acidosis was induced during the initial minutes of reperfusion. The work was later extended to include measurements of $[Na^+]_i$ during ischemia and reperfusion (122). These measurements directly demonstrated that $[Na^+]_i$ increases rapidly during ischemia and remains significantly elevated for 8–10 min after reflow. Several studies had previously shown complete recovery of pH_i within 30 s of reflow (e.g., Refs. 78, 88), thus verifying the idea that the fall in $[Na^+]_i$ lags behind the recovery of pH_i during early reflow. Tests of the specific involvement of Na^+-Ca^{2+} exchange were more elusive because of the lack of a selective pharmacological inhibitor. Nevertheless, functional recovery improves after reflow with high-sodium solution, a strategy designed to increase the transmembrane sodium gradient temporarily and thereby to blunt calcium influx via the exchanger (85). Direct measurements of intracellular calcium concentration during ischemia and reperfusion have verified that calcium overload does indeed occur, even with periods of ischemia as brief as 10–15 min (31, 96), and that

reperfusion brings about a further (albeit transient) exacerbation of the calcium overload (31).

In conclusion, the published work on pathogenesis implicates reperfusion-induced calcium overload in the mechanism of stunning, whereas the physiological studies point to a lesion at the level of the myofilaments.

Although cytosolic calcium concentration rises during ischemia (31, 96, 97), neither the impairment in myofilament calcium responsiveness (109, 160) nor the proteolytic degradation of the contractile protein machinery (47) has been found to occur during the ischemic phase. Thus reperfusion appears to be necessary for calcium overload to induce the mechanical abnormalities responsible for stunning. This may be due to the fact that the effects of calcium overload are prevented by the acidosis associated with ischemia, whereby the increased concentration of protons effectively competes with calcium for intracellular binding sites. The notion that increased proton concentration protects against the damage induced by calcium overload is supported by the finding that transient acidosis during early reperfusion can prevent myocardial stunning (78). The fact that the calcium overload occurring during ischemia is not sufficient to damage the contractile machinery underscores the concept that myocardial stunning is, in part, a manifestation of reperfusion injury (see sect. x).

2. Evidence for a role of calcium-activated proteases

The specific mechanisms whereby transient calcium overload undermines contractile protein function have not yet been pinpointed, but mounting evidence suggests a role for calcium-dependent proteolysis. Calcium-mediated injury in other systems is known to be mediated at least partly by calcium-activated proteases. These "calpains" are enzymes that cleave other proteins when cell calcium is elevated. They are widely distributed in cells from many tissues, including the myocardium (105, 107).

Several factors merit consideration in evaluating the potential pathophysiological relevance of calpains. The Michaelis constant of calpain I for calcium has been shown to be 1–20 μM *in vitro* (148). The calcium concentration required to activate calpains may be lower in intact cells due to the presence of membrane phospholipids and autolysis of calpains (149). Thus activation of calpain I, which comprises ~20% of the total calpain in rat myocardium, may require much lower calcium concentration in intact cells than in skinned cells. As reviewed in section VIII B1, myocardial stunning is always preceded by short-lived elevations of $[Ca^{2+}]_i$ that occur during ischemia and early reperfusion (31, 96), and the magnitude of the increase in $[Ca^{2+}]_i$ measured by NMR exceeds 1–3 μM . Despite being underestimated due to calcium buffering, this value nevertheless falls within the range of calcium concentrations required for activation of calpain I in

vitro (148). Calpains cause only limited proteolysis (i.e., they yield large protein fragments, not individual amino acids; Ref. 106). Thus it is not surprising that possible changes caused by calpains in stunned myocardium may not be visualized using conventional histological methods. For all these reasons, calpain I is a physiologically relevant candidate for intracellular myofilament proteolysis following ischemia and reperfusion.

The substrate specificity of calpain I with respect to cardiac myofibrillar proteins has not been fully characterized. Most of the information on myofilament substrate specificity of calpain derives from studies using calpain II (152). Nevertheless, DiLisa et al. (38) have shown that calpain I is very effective in digesting TnI and TnT in vitro. These observations are particularly noteworthy given the evidence (summarized above) implicating TnI proteolysis in the pathophysiology of stunning.

Direct exposure of cardiac myofilaments to activated calpain I reproduces the phenotype of stunned myocardium: maximal force is depressed, and sensitivity is blunted (49). These effects could be prevented by incubation with excess calpastatin, the natural inhibitor of calpain I (49). Thus these considerations make it at least plausible that calpain-mediated injury triggers selective proteolysis of the myofibrils, leading to postischemic contractile dysfunction.

To date, direct evidence that calpain I is activated in stunned myocardium is lacking. Yoshida et al. (166) have provided evidence that the activity of calpain I increases after ischemia-reperfusion in isolated rat hearts. Although the duration of ischemia was relatively long (45 min of zero-flow ischemia), a later study from the same group has shown that proteolysis of caldesmon, a cytoskeletal protein and a specific substrate of calpain I, occurs after periods of ischemia as brief as 10 min followed by 30 min of reperfusion (165). These studies suggest that calpain I may well be activated after brief ischemia followed by reperfusion.

If calcium-activated proteolysis is a key element of stunning, then it is logical to attempt to blunt the injury using calpain inhibitors. Such a strategy has been shown to be successful in isolated heart models, using various inhibitors (100, 159). Studies in vivo have not been performed but are eagerly awaited.

3. Possible role of sarcoplasmic reticulum dysfunction

Krause et al. (84) demonstrated that sarcoplasmic reticulum isolated from stunned myocardium had a decreased ability to transport calcium and postulated 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 via attenuated calcium release during systole. This hypothesis seems now implausible, because it implies

that the amplitude of the calcium transient is decreased, whereas in vitro data have shown that this is not the case (54, 86), as indicated above. Indeed, there is now ample evidence that calcium availability is not the limiting factor in stunning, at least in vitro (87).

4. Summary of the calcium hypothesis

The calcium hypothesis attractively explains many key features of the stunned myocardium (87).

1) Transient calcium overload has been implicated in the pathogenesis of stunning, and it is known that reactive oxygen species, which have been closely linked to stunning, can produce cellular calcium overload (36, 48).

2) Stunned myocardium is histologically benign; calcium-activated proteolysis, if it were to be partial and somewhat selective, need not visibly disrupt the sarcomere to alter function. Various thin filament proteins are notoriously susceptible to proteolytic degradation (8, 38, 157, 163); although the functional consequences have only just begun to be elucidated, direct exposure of the myofilaments to calpain reproduces the functional abnormalities of stunning (49).

3) The myofilament proteolysis hypothesis is entirely consistent with the observation that stunned myocardium remains responsive to inotropic stimulation, since the upstream mechanisms controlling $[Ca^{2+}]_i$ are presumed to be intact (94).

4) Reduction of myofilament calcium responsiveness rationalizes the remarkable efficacy of myofilament calcium sensitizers in the treatment of stunned myocardium (140).

5) Perhaps the most interesting feature of stunning is its eventual reversibility, with a distinctive time course of recovery over several hours or days. The proteolytic hypothesis puts forth a specific and testable rationale for slow reversibility: the partially degraded contractile proteins would have to be replaced by newly synthesized ones to repair the myofilaments, and the time courses for protein degradation and new protein synthesis are at least roughly appropriate (99, 104).

Despite these attractive features of the hypothesis, calcium overload and proteolytic myofilament injury may not underlie all forms of stunning. The most compelling evidence for this mechanism comes from isolated heart models of global ischemia and reflow. In other manifestations of stunning, such as the exercise-induced form (which can resolve fully within 1–2 h), it seems unlikely that proteolytic injury could occur and resolve so rapidly. Furthermore, the time course of recovery of contractile function after a brief coronary occlusion, with an early, rapid improvement in the first 1–2 h followed by a subsequent slower recovery (Figs. 3B and 5), suggests that repair of proteolytic damage may play a role in the late rather than early phase of dysfunction. Finally, it is im-

portant to recognize that the calcium hypothesis has yet to be tested extensively *in vivo*.

IX. INTEGRATION OF THE OXYRADICAL AND CALCIUM HYPOTHESES

Myocardial stunning is probably a multifactorial process that involves complex sequences of cellular perturbations and the interaction of multiple pathogenetic mechanisms. Much remains to be learned regarding this phenomenon, because none of the theories discussed herein explains the entire cascade of events that culminates in postischemic contractile abnormalities. For example, the origin(s) of reactive oxygen species as well as the mechanism whereby they induce mechanical dysfunction remain uncertain (12). Similarly, the exact nature of the myofilament lesion(s) that underlies impaired calcium responsiveness and the mechanism responsible for it are elusive. 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). For example, it is unknown whether the oxyradical hypothesis is applicable to exercise-induced stunning and stunning after subendocardium infarction and whether the calcium hypothesis is applicable to myocardial stunning *in vivo* (Table 2).

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 (12, 87). From a general perspective, there is abundant evidence to suggest a link between generation of reactive oxygen species and perturbed 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 (56). Furthermore, as discussed in section VIIA4, oxyradicals can cause dysfunction of the sarcoplasmic reticulum (132) and alter calcium flux across the sarcolemma (75–77, 130), causing cellular calcium overload. For example, it has been demonstrated that reoxygenation of cultured myocytes results in calcium overload, which can be greatly attenuated by antioxidant enzymes (111).

In the specific setting of myocardial stunning, considerable evidence has recently accumulated that strongly suggests a link between the oxyradical and the calcium hypotheses. Thus exposure of heart muscle to exogenously generated reactive oxygen species in the absence of ischemia reproduces many (but not all) of the phenotypic features of stunned myocardium (36, 48). As reviewed in section VIII, the fundamental lesion of excitation-contraction coupling in stunned myocardium appears to be a decrease of myofilament responsiveness to calcium. A similar decrease can be reproduced by exposing iso-

lated trabeculae to reactive oxygen species (48). A comparison of the effects of exogenously generated reactive oxygen species suggested that superoxide anion mimics the changes in excitation-contraction coupling more faithfully than does hydroxyl radical (48). It is not yet clear how these findings might converge to produce a unified view of stunning and its pathogenesis. Because exposure to exogenous reactive oxygen species produces calcium overload (36, 48), many of the effects of reactive oxygen species may reflect activation of calcium-dependent processes. However, reactive oxygen species could also directly damage the contractile proteins and impair their responsiveness to calcium (12). For example, exposure of myofibrils to exogenous oxyradicals has been found to impair ATPase activity via oxidation of thiol groups (150) and exposure to $\cdot\text{O}_2^-$ has been shown to impair maximal calcium-activated force (92), as detailed above. Clearly, more studies are required, particularly to examine the effects of reactive oxygen species (and antioxidants) on the myofilaments.

A unifying hypothesis for the pathogenesis of myocardial stunning is illustrated in Figure 6 (a detailed description of the postulated mechanisms is provided in the legend to Fig. 6). This paradigm is largely speculative but nevertheless encompasses the evidence available at this time and discussed in this review. According to this conceptual scheme (Fig. 6), oxyradical generation, calcium overload, and decreased myofilament calcium responsiveness can be viewed as different facets of the same pathogenetic mechanism, thereby reconciling the major current hypotheses of myocardial stunning (12, 87).

In summary, the pathogenesis of myocardial stunning has not been definitively ascertained. Thus far, the attention of experimentalists has focused mainly on the identification of individual mechanisms of injury. Three abnormalities (oxyradical generation, calcium overload, and impaired calcium responsiveness) have emerged as likely contributing factors (although not in all experimental preparations), and *in vitro* evidence suggests that they are interrelated. It is now essential to clarify the precise interactions among these three factors *in vivo* and the role that each of them plays in the various experimental settings of myocardial stunning.

X. IS MYOCARDIAL STUNNING A FORM OF REPERFUSION INJURY?

Both the oxyradical and the calcium hypotheses predict that a major portion of the injury responsible for myocardial stunning develops in the early phase of reperfusion (12, 87). This concept has been corroborated by extensive evidence. Studies in a canine model of myocardial stunning have shown that infusion of the antioxidant MPG attenuated postischemic dysfunction to a similar

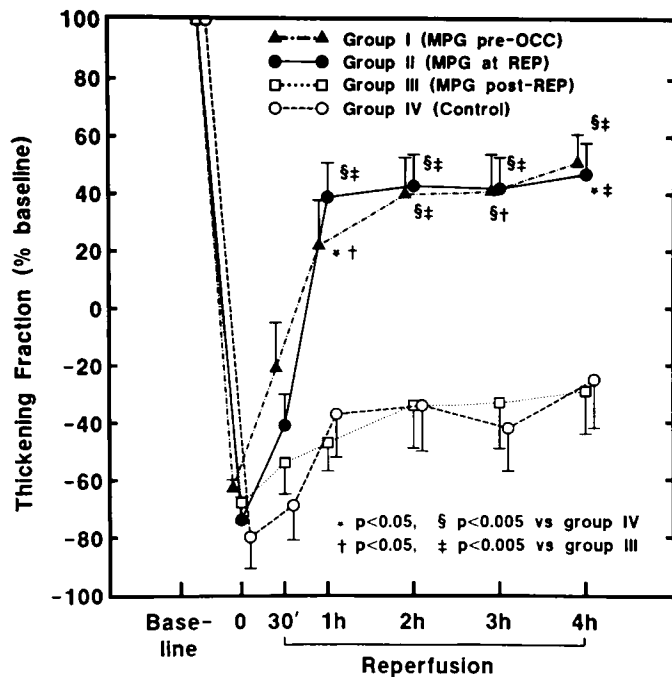


FIG. 11. Systolic thickening fraction in the ischemic-reperfused region 5 min after coronary occlusion (O) and at selected times after reperfusion in the following groups: *group I* (MPG infusion started 15 min before ischemia, $n = 8$), *group II* (MPG started 1 min before reperfusion, $n = 9$), *group III* (MPG infusion started 1 min after reperfusion, $n = 10$), and *group IV* (controls, $n = 10$). Myocardial stunning was produced in open-chest dogs by a 15-min coronary occlusion followed by reperfusion. Regional myocardial function was measured as systolic thickening fraction using a pulsed Doppler probe. Thickening fraction is expressed as percent of baseline values. MPG attenuated postischemic dysfunction to a similar extent irrespective of whether infusion was started before ischemia or just before reperfusion; however, infusion started 1 min after reperfusion was ineffective, suggesting that the critical radical-mediated injury occurs in the first few minutes after reperfusion. Data are means \pm SE. [From Bolli et al. (20), with permission. Copyright 1989 American Heart Association.]

extent whether the infusion was started before ischemia or 1 min before reperfusion; however, infusion started 1 min after reflow was ineffective (Fig. 11), suggesting that the critical radical-mediated injury occurs in the first few moments of reperfusion (20). Similar results were subsequently obtained with desferrioxamine (25). Furthermore, the spin trap, PBN, enhanced contractile recovery in open-chest animals even when the infusion was commenced 20 s before reflow; the magnitude of the protective effect was similar to that observed when the infusion was started before ischemia (24). That a substantial portion of the cellular damage responsible for stunning occurs immediately after reflow is further corroborated by direct measurements of free radicals (20–22, 24, 28a, 91, 135, 145) in the stunned myocardium, which have shown a burst in the initial moments after reperfusion (Figs. 2, 3A, and 4). Furthermore, if free radical production is inhibited during this initial burst, postischemic dysfunction is mitigated; however, if free radical production is

inhibited after the first 5 min of reperfusion (i.e., after the initial burst), no functional improvement is observed (Figs. 2 and 11) (20), suggesting that the free radicals important in causing myocardial stunning are those produced immediately after reflow. The observation that there is an initial recovery of function immediately after reperfusion, followed by a subsequent decline, also supports the occurrence of additional injury in the initial phase of reflow (93). In addition, modification of extracellular calcium at the time of reperfusion can markedly improve functional recovery (47, 88); it remains to be determined whether or not this mitigates free radical production or merely acts by reducing calcium overload.

In view of the above considerations, myocardial stunning appears to be, in part, a form of reperfusion injury, in the sense that it is caused in part by events that take place after restoration of flow (i.e., oxidant stress and calcium overload) (12). This concept may have significant therapeutic implications, because it suggests that therapies begun after the onset of ischemia can 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 myocardial stunning is not likely to be caused entirely by reperfusion injury. Indeed, interventions applied at the time of reperfusion (either designed to alleviate oxidant stress or aimed at preventing calcium overload) have generally failed to completely prevent myocardial stunning (12, 87). Even administration of “broad spectrum” antioxidant therapy (a combination of superoxide dismutase, catalase, desferrioxamine, MPG, and phenylalanine) has failed to completely prevent myocardial stunning, despite virtually complete suppression of $\cdot\text{OH}$ production (145) (Fig. 3B). Therefore, there appears to be a component of stunning that is not responsive to antioxidant therapy (no matter how vigorous) or to manipulations that alleviate postreperfusion disturbances in calcium homeostasis. This component is likely to be caused by derangements that occur during ischemia rather than after reperfusion. On the basis of these facts, it has been proposed (15) that the injury responsible for myocardial stunning consists of two components: 1) a component that develops during ischemia (ischemic injury) and 2) another component that develops after reperfusion (reperfusion injury) (Fig. 12). Judging from the effects of antioxidants or other therapies, the reperfusion injury component appears to be larger than the ischemic injury component (Fig. 12) (12).

It is also important to keep in mind that ischemic injury and reperfusion injury are not two independent entities. The term *reperfusion injury* is actually a misnomer, because it is ischemia that “sets the stage” for the development of reperfusion injury so that the appropriate term should be *ischemia-reperfusion injury* (15). As reviewed above, the studies that have directly measured

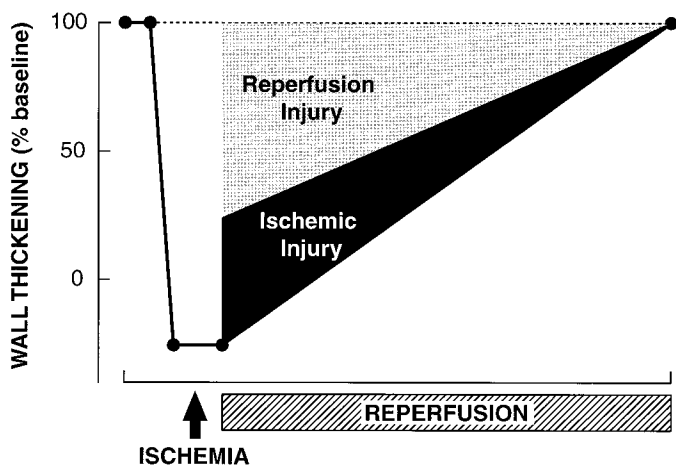


FIG. 12. Schematic representation of the two components of injury responsible for reversible posts ischemic contractile dysfunction (myocardial stunning). The changes in systolic wall thickening observed during and after a brief, reversible ischemic insult in experimental animals are illustrated. Note that during ischemia there is paradoxical wall thinning, followed by a slow recovery of wall thickening after reperfusion. On the basis of the available evidence, it appears that the injury responsible for myocardial stunning consists of two components: one that develops during ischemia (ischemic injury) and another (larger) that develops after reperfusion (reperfusion injury). Experimental data suggest that the magnitude of the reperfusion component is positively related to the magnitude of the antecedent ischemic component. Consequently, an intervention that alleviates the ischemic component of injury (e.g., adenosine, calcium antagonists, ATP-sensitive potassium channel openers) will also indirectly alleviate the reperfusion component, in absence of any direct effect on reperfusion injury [From Bolli (18a).]

free radicals in experimental models of myocardial stunning have found that the magnitude of the free radical generation after reperfusion was proportional to the magnitude of the flow deficit during the antecedent coronary occlusion (20–22, 24, 91) (and, by inference, to the severity of the antecedent ischemia; Ref. 22). These facts support the important concept (proposed in Ref. 134) that the severity of the reperfusion injury component of myocardial stunning is proportional to the severity of the ischemic injury component. Accordingly, any intervention that attenuates the severity of the ischemic injury will also, indirectly, attenuate the severity of the subsequent reperfusion injury (134). This is probably the reason why interventions that alleviate the injury incurred during ischemia [e.g., adenosine (71, 72, 134), calcium antagonists (39, 41, 58, 89, 119, 125, 154, 161), ATP-sensitive potassium channel openers (6)] are so effective in mitigating myocardial stunning, despite the fact that they have little or no direct effect on the reperfusion injury component of stunning. These interventions attenuate myocardial stunning by decreasing the ischemic injury component and, indirectly, the reperfusion injury component as well.

In summary, the available evidence suggests that myocardial stunning is caused in part by injurious events occurring during ischemia and in part by injurious events

occurring upon reperfusion (but triggered by ischemia). The relative proportions of these two components remain unknown and probably vary in different experimental settings. One issue that remains to be resolved is to discern how much of the injury responsible for stunning is caused by ischemia and how much by reperfusion.

XI. REPETITIVE STUNNING VERSUS HIBERNATION

Although myocardial stunning was originally described as a short-term phenomenon, it is theoretically plausible (and experimentally evident) that this depression of contractility may become protracted or even chronic. This concept adds a new, major dimension to myocardial stunning and has important implications for the pathophysiology of so-called chronic myocardial hibernation. Myocardial hibernation is a reversible ventricular dysfunction postulated to be secondary to a primary deficit of coronary flow (129). 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 chronic myocardial hibernation. 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 (10, 12, 28a, 35, 65, 117, 119, 133, 141, 146, 147). The notion that recurrent stunning can cause prolonged, reversible dysfunction has now been demonstrated (137). 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 (14, 137). The difference between repetitive stunning and hibernation is that regional perfusion is normal or near normal in the former but, according to the original postulate for this syndrome (129), abnormally low in the latter. Clinically, however, regional perfusion is rarely measured. On the other hand, 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 (14).

XII. PRECONDITIONING AGAINST MYOCARDIAL STUNNING

Can the myocardium be preconditioned against the development of stunning? To address this question, it is

important to distinguish between the two phases of ischemic preconditioning. The early phase, which develops within minutes from the ischemic stress and lasts 2–3 h, has been found to protect against myocardial stunning in the setting of multiple repeated ischemic episodes (28a, 155), as indicated in section IVB, but not in the setting of a single ischemic episode. With regard to the late phase of preconditioning, studies in conscious pigs (146, 147) and rabbits (153, 155) have demonstrated that this phase (which develops 12–24 h after the ischemic stress and lasts 3–4 days) provides significant protection against myocardial stunning (late preconditioning against stunning) (reviewed in Ref. 19). Thus an initial episode of stunning triggers a delayed adaptive response that renders the myocardium more resistant to subsequent episodes of stunning 24–72 h later. The development of late preconditioning against stunning is triggered by the generation of nitric oxide and reactive oxygen species and involves a complex signal transduction pathway that includes the ϵ -isoform of protein kinase C, protein tyrosine kinases, and NF κ B and ultimately leads to the upregulation of the inducible isoform of nitric oxide synthase, which generates the NO responsible for the protection (reviewed in Ref. 19).

XIII. FUTURE DIRECTIONS

Among the many issues that remain to be elucidated with respect to the mechanism and pathophysiology of myocardial stunning, the following appear to be particularly noteworthy.

1) Identification of the precise myofilament lesion responsible for stunning, of the mechanism that produces such lesion, and of the mechanism whereby such lesion leads to impaired calcium responsiveness is of the utmost importance for understanding fully the mechanism of this form of contractile dysfunction. Elucidation of the molecular basis of myocardial stunning would not only enhance our understanding of the pathophysiology of ischemic heart disease but would also lay the groundwork for better preventive therapies.

2) With regard to the oxyradical hypothesis, major unresolved issues are the exact sources of the free radical species responsible for stunning, the relative contributions of different radical species to this phenomenon, and, perhaps most importantly, the precise mechanism whereby a short burst of oxyradical generation results in prolonged depression of contractile dysfunction.

3) With regard to the calcium hypothesis, it is important to determine whether calcium overload and impaired calcium responsiveness play a role in the genesis of myocardial stunning *in vivo*.

4) The mechanism of myocardial stunning after prolonged, partly reversible regional ischemia (subendocar-

dial infarction) and after exercise-induced ischemia remains enigmatic. Investigation of these forms of stunning is in order.

5) Finally, it is now clear that brief ischemia resulting in stunning is associated with changes in gene expression, which in turn lead to changes in cardiac phenotype, particularly after recurrent brief ischemic episodes. The changes in gene expression associated with stunning may underlie the dedifferentiation of myocytes noted in hibernating myocardium, late preconditioning against ischemia, and a number of other potentially important long-term processes. This area warrants further characterization.

XIV. SUMMARY

Despite considerable progress, the pathogenesis of myocardial stunning has not been definitively deciphered. Among the numerous mechanisms proposed, three have emerged as likely contributing factors: 1) generation of oxygen radicals, 2) calcium overload, and 3) decreased responsiveness of contractile filaments to calcium. It is now generally accepted that oxygen-derived free radicals play an important role in the pathogenesis of several forms of myocardial stunning. In the setting of a single 15-min coronary occlusion, mitigation of stunning by antioxidants has been reproducibly observed by several independent laboratories in different species. Similar protection has been demonstrated in conscious animals, *i.e.*, in the most physiological experimental preparation available. Furthermore, generation of free radicals in the stunned myocardium has been directly demonstrated both by spin-trapping techniques and by aromatic hydroxylation, and attenuation 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, in part, as a sublethal form of oxyradical-mediated reperfusion injury. Nevertheless, it remains unclear whether oxyradicals play a role in all forms of stunning. There is presently no evidence that they contribute to exercise-induced postischemic dysfunction. Data regarding the role of oxyradicals in myocardial stunning after a prolonged (>20 min) ischemic insult, causing subendocardial infarction, are conflicting. Substantial evidence also supports the idea that calcium overload during reflow triggers myofilament dysfunction, which effectively uncouples excitation from contraction so that for any given calcium transient, the myocardium

generates less force. In this sense, myocardial stunning could be viewed as a disturbance of myofibrillar function. However, it is not yet clear whether or not myofibrillar alterations are a general feature of all forms of stunning.

The oxyradical hypothesis and the calcium hypothesis are not mutually exclusive and, in fact, may represent different steps of the same pathophysiological cascade. For example, generation of oxyradicals may cause calcium overload, and both of these processes could lead to damage and dysfunction of myofibrils. The major challenge in the next few years will be to unravel the molecular mechanisms whereby a brief episode of ischemia can cause such a prolonged period of contractile dysfunction. Given the heterogeneity of postischemic dysfunction, an important area for future investigations will be the precise identification of the role that oxyradical generation, calcium overload, and myofibrillar dysfunction play in the various experimental settings of myocardial stunning.

Myocardial stunning is likely to occur commonly in patients with coronary artery disease and to contribute significantly to the morbidity associated with this disorder. It is hoped that the concepts discussed in this article will provide not only a conceptual framework for further investigation of the pathophysiology of reversible ischemia-reperfusion injury, but also a rationale for developing better diagnostic modalities and new therapeutic strategies designed to prevent postischemic ventricular dysfunction in humans.

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