EDITORIAL COMMENT

Cellular and Extracellular Mechanisms Causing Myocardial Stunning*

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Myocardial stunning involves a profound and slowly recovering decrease in contractility that follows a brief but severe episode of ischemia (1). A report in this issue of the Journal of the American College of Cardiology (2) challenges the prevailing view that the mechanisms underlying stunning are cellular in origin. Interestingly, Chandrashekhar et al. (2) found there was little difference in the contraction of isolated myocytes removed either from stunned or from control hearts. Furthermore, Ca\(^{2+}\) handling and Ca\(^{2+}\) responsiveness were similar in myocytes from stunned or control hearts. Since stunning did not appear to be manifest at the cellular level, they concluded that the mechanisms underlying stunning are extracellular. This conclusion is provocative, since it conflicts with a large number of other studies that suggest that the mechanisms of stunning are cellular in origin. However, it must be acknowledged that Chandrashekhar et al. (2) do systematically and directly assess the function of stunned hearts at the cellular level. In contrast, almost all previous studies that infer a cellular basis for the mechanisms of stunning have made this inference from studies of multicellular preparations. Thus, the study by Chandrashekhar et al. (2) raises the possibility of a largely unexplored alternative source for contractile dysfunction with myocardial stunning. An extracellular mechanism for myocardial stunning could have important implications for developing therapeutic approaches for this ischemic injury. In this editorial, the work of Chandrashekhar et al. will be considered in the context of previous work by others.

Myocardial stunning has been well documented using various animal models where myocardial blood flow can be controlled or measured and ventricular function evaluated (3). In humans, however, a diagnosis of stunning is limited by the difficulty of demonstrating reduced cardiac function in the setting of a restoration of normal blood flow. Nevertheless, myocardial stunning is thought to occur in humans after a brief interruption of myocardial blood flow under conditions such as acute infarction with early reperfusion, unstable angina, cardiac surgery and transplantation (4). Thus, although the existence of myocardial stunning is well established in animal models and thought to be clinically relevant in humans, the mechanisms that underlie stunning continues to be a subject of investigation and debate (5).

Studies of myocardial stunning have followed a reductionist approach of using simplified experimental models. Models have been used with various levels of complexity ranging from intact animals to isolated cells. In contrast with more intact systems, use of simplified experimental systems offers greater control over experimental variables, and the ability to investigate detailed mechanisms. However, a perennial concern is whether simplified experimental systems truly reflect physiologic mechanisms in the intact organism. Many studies, including that of Chandrashekhar et al. (2), are limited by this concern, which makes assessment of underlying mechanisms uncertain.

Studies of increasingly simplified experimental models of stunning are briefly surveyed in the following discussion. As noted previously, stunning is difficult to assess in humans. In contrast, stunning has been well documented in conscious animals and in anesthetized animals (reviewed in [3]). Stunning was found to be ameliorated with use of a calcium antagonist (6) and antioxidant (7). These and other studies have suggested that with ischemia and reperfusion there is calcium overload and increased levels of reactive oxygen species which ultimately cause injury to the heart.

A simpler, more controllable, though still relatively intact experimental system is the isovolumic perfused Langendorff heart, where an experimental model of stunning has been developed consisting of global ischemia followed by reperfusion. Previous studies found that in the perfused heart stunning was associated with a reduction of developed pressure (due to a marked elevation of end-diastolic pressure along with a reduction of systolic pressure). The roles of calcium and oxygen free radicals as potential mediators of stunning has also been confirmed in the perfused heart (8–11). However, from these studies it has not been clear if the causative mechanism operates on intracellular or extracellular components. Measurements of Ca\(^{2+}\) in perfused hearts demonstrated that stunning was not due to a decrease in the levels of activator Ca\(^{2+}\), suggesting a role for decreased Ca\(^{2+}\) sensitivity (12).

Further simplification of the experimental model of stunning was achieved by using trabeculae dissected from stunned hearts. Use of trabeculae offers several advantages for study of myocardial function and investigation of physiologic mechanisms. The geometrically simple trabecula preparation allows precise estimation of mechanical function; sarcomere lengths can be measured and controlled.

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(13), and the diffusion of small molecules through gap junctions allows spread of fluorescent indicators to the cytosol of all myocytes in the trabeculae after iontophoretic loading of indicator to a single cell (14). Notwithstanding the simplicity of this preparation, trabeculae still retain important elements of the myocardium, such as cell to cell cytoplasmic and electromechanical coupling, cell–matrix coupling and the presence of nonmyocyte cells (15).

A series of landmark studies from Marban’s laboratory strongly suggested that stunning involves cellular mechanisms. In trabeculae from stunned hearts there was little change in the Ca$^{2+}$ transient, but there was a marked decrease in Ca$^{2+}$ responsiveness, which consisted of a decrease in the maximum Ca$^{2+}$-activated force and a requirement for higher Ca$^{2+}$ levels to achieve half maximal activation of force (16). Consistent with a role for oxygen free radicals in stunning, the effects of stunning could be partially mimicked by agents that exogenously generated oxygen free radicals (17).

The trabecula preparation can be further simplified by permeabilizing the cell membranes (skinning) to study myofilament function without the excitation–contraction coupling system or soluble cytoplasmic factors. These studies showed that the decreased Ca$^{2+}$ sensitivity found in stunned trabeculae was also found after skinnning, suggesting that decreased Ca$^{2+}$ sensitivity with stunning is an intrinsic myofilament property (18). Furthermore, the effects of stunning could be reproduced by a Ca$^{2+}$-activated protease (18), suggesting that stunning involves proteolytic damage to the myofilaments. Consistent with this, stunning was shown to involve degradation of the troponin subunit TnI, and both stunning and TnI degradation could be prevented by low Ca$^{2+}$ conditions (19). Finally, TnI degradation could be mimicked in skinned fibers by a Ca$^{2+}$-activated protease (19). Studies in other laboratories have now confirmed TnI proteolysis after stunning (20,21), suggesting that TnI proteolysis may play an important role in the pathophysiology of stunning (20–22). Taken together, these studies suggest that stunning involves generation of free radicals, Ca$^{2+}$ overload and damage to the myofilaments by Ca$^{2+}$-activated proteases. However, whether these are sequential steps in an injury process or are separate events that contribute to injury is not clear.

As noted above, with a simplified experimental system, differences from more intact systems may be a limitation. For example, stunning in the whole perfused heart results in considerable elevation of diastolic pressure; however, in trabeculae, the diastolic force is almost unchanged (16). This difference indicates that a feature of the response of the intact heart to stunning has been lost in the simpler trabecula preparation. This difference emphasizes the need for caution when extrapolating from simpler to more complex systems.

Chandrashekhar et al. (2) have used a very simple experimental model for stunning, namely the single intact myocyte. A strength of their approach is the attempt to directly assess the role of cellular versus extracellular mechanisms. There has been only one previous study of cells isolated from stunned hearts (23). Lew et al. (23) found stunning resulted in some intrinsic defects at the cellular level (decreased cell shortening and duration of shortening and increased cell length), but other aspects of cell function were unaffected (rates of shortening and relengthening).

Chandrashekhar et al. (2) used a well established protocol to produce myocardial stunning in an isovolumic Langendorff perfused rat heart. This protocol (20 min ischemia followed by 20 min reperfusion) does not cause significant myocyte necrosis (9,24). After this protocol, myocardial stunning was evident from an appreciable decline in left ventricular developed pressure. The mechanical function of cells isolated from these hearts was assessed from measurements of cell shortening, and excitation–contraction coupling was assessed from measurements of Ca$^{2+}$ transients using fura-2. The major findings were that the reduced developed pressure of the whole heart with stunning could not be explained on the basis of a reduction in myocyte contractility or impaired myocyte Ca$^{2+}$ handling or Ca$^{2+}$ sensitivity. Chandrashekhar et al. (2) therefore concluded that the site of injury with stunning must lie outside of the myocyte. If the mechanisms of stunning are not cellular, what then might be the extracellular mechanisms causing stunning? Chandrashekhar et al. (2) suggest there may be defects in the extracellular matrix or abnormalities in the coupling between myocytes and the interstitium. In addition stunning could involve abnormalities in myocyte to myocyte electromechanical coupling. Stunning does not involve impaired electric activation of the heart (25).

How can this new study by Chandrashekhar et al. (2) be reconciled with the compelling body of previous work suggesting stunning arises due to alterations at the cellular level? A simple reconciliation could be that both cellular and extracellular mechanisms are involved in stunning. Indeed, stunning has been shown to involve structural damage to both the extracellular matrix (11,26) and the myofilaments (16–22). However, Chandrashekhar et al. (2) found no evidence of functional impairment at the cellular level with stunning, leading to the conclusion that all of the injury with stunning was extracellular. If stunning is entirely due to extracellular mechanisms, it is difficult to see how some previously reported manifestations of stunning can be accounted for. For example, how could alterations in the mechanical properties of extracellular structures account for decreased Ca$^{2+}$ sensitivity in trabeculae from stunned hearts?

Because Chandrashekhar et al. (2) report a negative study, further work is necessary to support an extracellular hypothesis for stunning. In particular, it would be important to establish that some extracellular property was indeed perturbed by a stunning protocol, and further that such a perturbation could account for the mechanical manifestations of stunning.
Since the findings of Chandrashekhar et al. (2) are so intriguing, and also perhaps unexpected given previous work by others, careful consideration must be given to the functional properties of cells isolated from hearts. This again relates to the concern with the ability of simplified experimental systems (in this case single cells) to adequately reflect stunning in more intact systems. For example, it is possible that the myocyte isolation procedure is injurious. Thus, the effects of stunning could be masked by injury to myocytes during myocyte isolation. Moreover, functional impairment due to stunning could even be protective (in terms of preconditioning) during myocyte isolation. According to this, myocytes from stunned hearts might be expected to perform even better than myocytes from control hearts. Consistent with this, Chandrashekhar et al. (2) did find that when myocytes were challenged by increased Ca\(^{2+}\) levels or mechanically loaded by increased solution viscosity, cells from stunned hearts did perform appreciably better than cells from control hearts.

In conclusion, several studies now suggest the hypothesis that impaired myocardial function with stunning is due to alterations in the myofilaments. Now Chandrashekhar et al. (2) suggest an alternative hypothesis that stunning arises from extracellular mechanisms. Which of these hypotheses, the cellular versus the extracellular, prove to be true awaits further study. The work by Chandrashekhar et al. (2) is important for suggesting an alternative mechanism for myocardial stunning. This suggestion should act as an impetus for further work to investigate the mechanisms involved in stunning.

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