Editorial Comment

Hemodynamic Overload and the Regulation of Myofibrillar Protein Degradation

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The growth and hypertrophy of left ventricular myocardium in response to pressure or volume overload or segmental loss of functional myocardium was once considered a purely beneficial, structural adaptation to reduce or normalize systolic and/or diastolic wall stress. This concept has been challenged, however, by a growing body of evidence indicating that most forms of left ventricular hypertrophy (LVH) are associated with increased morbidity and mortality caused by progressive left ventricular contractile dysfunction, irreversible muscle injury, and the ultimate development of congestive heart failure. Thus, a rational approach to the patient with structural heart disease associated with LVH requires a clear understanding of the underlying metabolic processes responsible for the growth of cardiac muscle in response to hemodynamic overload and the resultant changes in muscle structure that may be responsible for this progressive functional deterioration. Despite more than 25 years of laboratory investigation, however, the complex cellular and molecular mechanisms responsible for converting mechanical signals into changes in myofibrillar protein synthesis and turnover are only now being unraveled. In this issue of Circulation, Magid and colleagues provide new and interesting information about the relative contributions of these two opposing metabolic processes in the progressive hypertrophy and dilatation that occurs with acute and chronic aortic regurgitation.

Myofibrillar Protein Synthesis and Turnover

Myofibrillar proteins consist of a group of structural and catalytic proteins that compose the myofibril, the contractile organelle of the cardiac muscle cell. Taken together, these 10 or more proteins account for approximately 40% of left ventricular dry weight. Alterations in cardiac muscle mass in response to altered loading conditions are largely accounted for by changes in the amounts of these protein subunits (and thus the number and size of myofibrils). Myofibrillar proteins, like all other intracellular proteins, are in a dynamic state of continual degradation and resynthesis. The balance between these opposing processes ultimately determines the number of functional contractile units within each cardiac myocyte. Numerous studies in many model systems have shown that imposition of acute stretch or increased workload on cardiac muscle in vitro or in vivo improves nitrogen balance and augments myofibrillar protein synthesis (for review, see Reference 3). In this respect, the study by Magid et al2 confirms previous studies by Moalic et al3 that showed a substantial increase in myocardial protein synthesis after the induction of aortic regurgitation in the rat. Conversely, removal of the causal mechanical stimulus reduces rates of myofibrillar protein synthesis in ventricular myocytes and decreases muscle cell mass.4-9 The regulation of myofibrillar protein synthesis in response to mechanical factors appears to involve transcriptional as well as translational mechanisms of gene regulation and may involve effectors and second messengers common to growth factor and neurohumoral signal transduction pathways activated during other forms of muscle growth.3,10,11

In contrast to the clear correlation between mechanical load and rates of myofibrillar protein synthesis, the effects of altered loading conditions on myofibrillar protein degradation are considerably less certain. Some of this uncertainty relates to general methodological problems in assessing protein turnover rates by use of isotopic tracers in vivo and in vitro and to physical and kinetic compartmentation of these long-lived proteins within myofibrils.12 Despite these shortcomings, a general consensus exists to indicate that rates of total protein degradation (and often by inference the degradation of contractile proteins like actin and myosin) vary in response to mechanical unloading in a pattern directionally opposite to that of protein synthesis. That is, decreased hemodynamic load results in “disuse” atrophy caused by increased rates of myofibrillar protein degradation and decreased rates of protein synthesis.7-9 The situation encountered during various experimental models of muscle hypertrophy is more problematic, since protein degradative rates measured in vivo and in vitro have been found to be increased, decreased, or unchanged compared with muscles not undergoing compensatory hypertrophy in response to acute hemodynamic overload. Thus, a unifying role for protein degradation in the hypertrophic process has not been proven.

In this regard, the study by Magid et al2 provides the first indication that reduced myofibrillar protein degradation may be a major contributing factor to the progressive cardiac enlargement and dilatation during the

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chronic phase of LVH progression in rabbits with aortic regurgitation. These investigators have successfully applied an indirect approach to estimate the fractional degradative rate of the mixture of all left ventricular proteins, and more importantly, myosin heavy chain (MHC), a myocyte-specific contractile protein that accounts for approximately 20% of the total protein content of left ventricular myocardium. Using well-established methods of tracer amino acid administration to accurately measure protein synthesis in the intact experimental animal, they used myocardial mass changes assessed by echocardiography to indirectly estimate protein degradative rates as the difference between rates of synthesis and growth. This indirect approach has been widely used to estimate protein half-lives in myocardial and other tissues, since there are few (if any) reliable methods to directly assess individual protein degradative rates in vivo. Their results were straightforward (despite the complex mathematical analysis involved in the derivation of protein turnover rates) in that MHC fractional degradative rates (Kd) were considerably reduced 1 month after the induction of moderate aortic reduction compared with sham-operated control animals. In other words, the apparent half-life of MHC in the sham-operated animals (approximately 7 days) was considerably prolonged in chronic aortic regurgitation (approximately 10.5 days).

Even a small prolongation in MHC half-life (in the absence of a major decrease in the rate of MHC synthesis) would eventually produce a significant accumulation of this protein over time. Magid et al. concluded that this reduced susceptibility of MHC (and probably other myofibrillar proteins) to intracellular proteolysis observed many weeks after induction of aortic regurgitation was a causative factor for the progressive LVH observed in these animals. This conclusion seems warranted, barring major methodological problems associated with measuring the rate of synthesis of an individual protein in vivo. Also of interest, however, is the possibility that prolongation of myofibrillar protein half-lives might in some way contribute to the progressive contractile dysfunction observed in patients with chronic aortic regurgitation. Although this is an attractive hypothesis, proof will require a much better understanding of the regulation of myofibrillar protein degradation in the normal as well as the hemo-dynamically stressed ventricular muscle cell. It is likely, however, that future studies into the cellular mechanisms involved in regulating myofibrillar protein degradation will require studies performed in less complex model systems.

Cellular Mechanisms Involved in Coupling Mechanical Stimuli to Alterations in Myofibrillar Protein Degradative Rates

Primary cultures of neonatal and adult cardiac myocytes subjected to passive stretch and active tension development have provided useful tools that ideally will improve our understanding of the cellular mechanisms involved in myofibrillar protein degradation. These in vitro systems have distinct advantages over the in vivo situation or the use of isolated, intact muscles maintained by tissue perfusion or superfusion. In cell culture, mechanical signals can be accurately defined and controlled. The use of highly enriched primary cultures of ventricular muscle cells ensures that mechanical stimuli act directly on the muscle cell population rather than on other mechanosensitive cells within muscle tissue. Finally, myocytes can be maintained in culture for many days in nutrient-rich, defined media, and their viability can be easily monitored. The use of isolated perfused or superfused tissues to assess protein degradative rates during brief mechanical loading/unloading has very limited utility in the study of the cellular and molecular events of myofibrillar protein degradation because of the relatively long turnover rates of these proteins compared with the limited viability of these in vitro preparations.

Using cultured embryonic chick muscle fibers, Strohman and coworkers first demonstrated that mechanical factors had a direct impact on the accumulation of MHC and other myofibrillar proteins through a posttranslational mechanism that operates at the level of protein degradation. We have recently confirmed and extended their observations in highly enriched, spontaneously contracting cultures of rat ventricular myocytes maintained in serum-free medium. In our studies, both potassium depolarization and blockade of electrical depolarization with different calcium channel blocking agents reduced MHC accumulation and led to a time-dependent disappearance of intact myofibrils. These effects were entirely reversible, and resulted in part from a marked increase in the rate of MHC degradation. In addition, Vandenburg and coworkers have shown that sustained or cyclic stretch of spontaneously contracting, embryonic chick skeletal muscle myotubes decreased the degradative rate of long-lived proteins compared with unstretched, spontaneously contracting control cells. Preliminary results from our laboratory also indicate that cyclic stretch of verapamil-treated, quiescent cardiac myocytes prevents the proteolysis-dependent disappearance of intact myofibrils in these arrested cells (D. Simpson, W. Sharp, L. Terracio, T.K. Borg, and A.M. Samarel, unpublished observations). Thus, it is likely that mechanical activity, rather than electrical activity, in some way mediates the susceptibility of myofibrillar proteins to intracellular proteolysis in the absence of exogenous neural or humoral factors.

Although cellular “stretch” and active tension development affect myofibrillar protein degradative rates in these cell culture models, the manner in which mechanical deformation is converted into biochemical signals leading to altered proteolytic susceptibility remains unclear. In a recent review, Vandenburg outlined some of the putative “mechanogenic” second messengers involved in altering cell growth in a variety of mechanically sensitive cells, including striated muscle cells. These transducing pathways are of two general types: extracellular matrix (ECM)–integrin–cytoskeleton complexes and plasma membrane–associated molecules involved in the production of “diffusible” second messengers common to other receptor-mediated signal transduction pathways. These mechanical signals may be transduced directly by deformation activation of mechanosensitive catalysts or may be mediated by deformation of the closely associated ECM–integrin–cytoskeleton complex. Putative mechanosensitive “receptors” include stretch-activated and inactivated ion.
channels, membrane phospholipases, and adenylyl cyclase. The second messengers include cyclic AMP, inositol phosphates, prostaglandins, diacyl glycerol, and intracellular Na\(^+\) and Ca\(^{++}\), all of which have been implicated in neurohumorally mediated growth responses in other cell types. These putative second messengers are then capable of activating effector systems including protein kinases A and C and Ca\(^{++}\) calmodulin–dependent protein kinases that may be involved in the early events in cellular growth. Although a number of signaling molecules have been implicated in the regulation of myofibrillar protein gene expression in response to hemodynamic overload, the pathways linking mechanical signals to changes in cardiac myocyte protein degradative rates are not known.

**Does the State of Myofibrillar Assembly Indirectly Regulate the Susceptibility of Myofibrillar Proteins to Proteolysis?**

It is difficult to envision exactly how hemodynamic overload (or for that matter its generated second messengers) might directly alter the susceptibility of an individual contractile protein to proteolysis. We have shown that the accelerated MHC degradation in arrested ventricular myocytes was not suppressed by chloroquine, leupeptin, or E-64, agents capable of inhibiting a variety of lysosomal and nonlysosomal proteinases. Thus, it seems reasonable to conclude that mechanical forces alter myofibrillar proteolytic susceptibility via a rate-limiting step proximal to degradative processing. Strohman and coworkers originally hypothesized that myofibrillar assembly and stability requires association of newly synthesized subunits with cytoskeletal components at or near the plasma membrane. Interference with the assembly of these newly synthesized proteins by disruption of the ECM–integrin–cytoskeletal complex during mechanical unloading would secondarily make the unassembled myofibrillar proteins more susceptible to the action of muscle cell proteolytic processes. In this way, the process of myofibrillar protein assembly and disassembly could indirectly regulate the turnover of these structural proteins. Similarly, mechanical overload (or its generated second messengers) might shift the balance between assembled and unassembled myofibrillar protein subunits by decreasing the probability that subunits are disassembled and translocated to cellular sites where proteolysis normally occurs. Mechanical forces might also activate an effector system that directly modulates myofibrillar assembly/disassembly via a posttranslational phosphorylation or other modification of a key structural element. The transcription of a critical intermediate required for proper folding or integration of contractile proteins into myofibrils might also be affected by mechanosensitive signals arising during cellular deformation. In summary, it is likely that one or more of these potential pathways are operating to indirectly suppress myofibrillar protein degradation in Magid’s chronically volume-overloaded rabbit hearts.

**Future Directions**

It should be evident from this discussion that a clear understanding of the regulation of myofibrillar protein degradation in response to mechanical as well as other stimuli is not yet at hand. Despite the fact that all of the major myofibrillar protein genes have been cloned and sequenced, we still do not know exactly what structural features of these highly conserved proteins are critical for myofibrillar assembly and stability. However, the advent of several new approaches, including the ability to express native and mutated myofibrillar proteins in homologous and heterologous cell systems and improvements in the design of mechanical cell stimulators, should provide additional useful tools to aid in the search for an answer to this perplexing problem in cardiac muscle metabolism.

**References**


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