Giant Molecule Titin and Myocardial Stiffness

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The beauty of the new titin hypothesis is that the heart is able to change its resting length-tension relationship under chronic stress conditions by isoform switching, thereby rehabilitating the old clinical paradigm of “diastolic tone.” The path from hypothesis to reality is rocky, however, as the articles by Wu and Neagoe in this issue of Circulation show.1,2

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Wu et al1 found that the titin isoforms N2BA/N2B ratio was decreased from a normal ratio of 1.0 to 0.8 in the paced dog heart. Because N2B represents the isoform with stiffer elastic properties, this resulted in an increased passive stiffness in isolated muscle strips. The authors suggest that this “titin-based” stiffness is acting in concert with “collagen-based” stiffness and that it may counteract ventricular dilatation in canine hearts failing because of chronic pacing.

The results are somewhat in contrast with an earlier study by the same group in which a switch to the more extensible isoform N2BA was shown to occur in the subendocardium of canine hearts after 2 weeks of pacing. This seemingly contradictory result might reflect a different time course of titin isoform expression.3

Neagoe et al4 report a titin isoform shift from 30:70 (ratio 0.42) of N2BA/N2B in normal human myocardium to 47:53 (ratio 0.89) in nonischemic regions from human hearts transplanted because of coronary artery disease (CAD). Total passive tension was reduced in CAD compared with control, indicating a more compliant behavior of the myocardium. The authors, referring to the well-known increase in cardiac stiffness due to collagen in chronic heart failure, hypothesize that this isoform shift occurs to counteract the global stiffening caused by chronic preload elevation in CAD patients.

Titin and Collagen

The giant molecule titin, the “third sarcomeric filament system,” is the third most abundant (10%) of the cardiac proteins, after myosin (40%) and actin (22%). Spanning as one molecule from the Z-line to the M-band, it is indispensable in the structural integrity of developing and mature sarcomeres.4 Titin provides elasticity to the sarcomere and permits elastic recoil. Titin’s function is adapted by different isoforms (alternative splicing) to the specific needs of striated muscle. The most variable regions of the whole molecule represent the molecular spring of the sarcomere localized in the I-band. In cardiomyocytes, 2 titin isoforms, N2B and N2BA, are coexpressed in each sarcomere in a species-specific ratio. For example, N2B, the smaller and stiffer isoform, has been shown to be exclusively expressed in rat and mouse ventricles, whereas N2BA is the dominating titin found in bovine atria. Most species, however, possess a mixture of both isoforms.5 In addition to the titin-based regulation of myocardial stiffness that is important at low to intermediate sarcomere lengths, collagen-based stiffness plays a major role at sarcomere elongation.

The fibrillar collagen network consists mainly of collagens I and III, which are coexpressed in a constant ratio. It is important to the mechanical properties of tissue because it builds a scaffold to keep the different cellular and noncellular compartments in order. Under certain physiological conditions, the collagens contribute to a small extent to the regulation of diastolic stiffness and sarcomere length (rung as wavy chords, they prevent sarcomere lengths over 2.3 μm), but they provide myocyte lateral alignment.6

Cardiac pathology is usually concerned with an increased content, ie, fibrosis, or an inappropriate degradation of collagen. This will influence left ventricular (LV) function in a variety of fields, including diastolic stiffness, nutrition, impulse propagation, arrhythmia, and dilatation resulting finally in depressed systolic function. It is important to note that quantitative changes in collagen expression are usually accompanied by a switch in collagen type and arrangement; this should be taken into consideration when describing function-structure relationships. Increased collagen will lead to an increased passive tension and myocardial stiffness.7

LV Chamber Stiffness

The pacing-induced tachycardia model of heart failure produces a dilated cardiomyopathy with little or no change in LV mass. Over a period of several weeks, the end-diastolic volume/mass (or radius/thickness) ratio increases, and despite substantial remodeling and enlargement of the ventricle, which might suggest a decrease in chamber stiffness, most studies indicate that the slope of the end-diastolic pressure-volume curve remains normal or near normal. The stiffness of a unit of the LV wall (defined by myocardial stress-strain relations) has been found to be normal by some investigators and increased by others. It is difficult to reconcile these disparate findings except to say that different experimental models and different periods of pacing might be responsible.8–10 Unfortunately, Wu et al1 did not correlate their findings with measurements of ventricular volume, mass,
function, or structure in their pacing model. Changes in the stiffness of the ventricle and the myocardium are thought to be mediated largely by alterations in the collagenous matrix that surrounds the cardiomyocyte. Wu et al. suggest that the large intrasarcromeric protein titin also plays a role in determining ventricular stiffness.

It should be emphasized, however, that Wu et al. stated that increased passive tension of the myocardium from failing hearts was the result of a “coordinated change in both collagen and titin, with titin dominating at short to intermediate sarcomere lengths and collagen at long sarcomere lengths.” Because end-diastolic sarcomere lengths in failing hearts are likely to be intermediate to long, LV size and geometry must be affected primarily by the collagenous matrix, with titin playing a lesser role.

The results of any study of the physical properties of the ventricle are likely to be influenced by the nature of the experimental model of heart failure as well as the duration of the pathophysiological process. It is possible that the overall effect of many interdependent and interacting factors is to facilitate a dissolution of the extracellular matrix and allow an early chamber dilatation, which is similar to the result seen in mitral regurgitation. During this change in ventricular geometry, titin limits sarcomere overstretch. Later, a “coordinated change in both collagen and titin” might act to limit further ventricular dilatation.

The study by Neagoe et al., though carried out in human hearts, is hampered by the fact that patients with CAD represent an extremely variable cohort. The fact that hemodynamic data were available from only 2 CAD patients makes it difficult to draw definitive conclusions as to changes of chamber stiffness. The authors tried to compensate for the lack of clinical information by providing data on cardiac troponin J modifications as an indicator of preload. This, however, is extremely artificial because this information was obtained from short-time ischemia experiments in Langendorff perfused isolated rat hearts, a situation far removed from that in ischemic human hearts. Nonischemic regions from CAD hearts were found to show decreased total muscle strip stiffness, which is proposed to counteract the global stiffening caused by chronic preload elevation.

The functional consequences of an increased ventricular stiffness include a limitation in the degree of enlargement of the failing heart. This limitation protects the ventricle against serious rises in systolic wall stresses and thereby attenuates the downward spiral of progressive afterload excess and systolic dysfunction. On the other hand, it should be recognized that chamber dilatation provides a mechanism for maintaining an adequate stroke volume in the presence of a low ejection fraction. This is in contrast to the “nonstructural dilatation,” which was described as the consequence of volume overload without renin-angiotensin-aldosterone system activation. Excessive stiffness may actually prevent dilatation and result in a decline in stroke volume. Thus, an early chamber enlargement may be a beneficial adaptation to depressed myocyte and ventricular contractile function. Later, a stiffening effect might prevent progressive ventricular dilatation, but excessive stiffening is maladaptive.

**Pacing-Induced Failure**

The model of chronic high-rate pacing in dog or pig hearts seems to resemble the situation in failing human hearts, but its experimental period is short and the structural phenotype is most probably different.

In failing human hearts with dilated cardiomyopathy, a considerable amount of fibrosis is present (up to 40% versus 10% in normal human hearts). Furthermore, myocyte degeneration with reduction of contractile material and titin occurs, and cell death and loss of myocytes are common. This may leave the titin/myosin heavy chain quotient seemingly unaltered, when in fact absolute values may be significantly reduced.

In pacing-induced dilated cardiomyopathy with heart failure, the structural situation is not clear. One group reported massive loss of myocytes and significant replacement fibrosis, whereas others observed either a reduction of the collagen fraction or collagen nonuniformity. Weber et al. concluded that ventricular dilatation and failure after pacing are on the basis of an architectural remodeling of the myocardium, termed “structural dilatation.” Wu et al. did not report any changes in LV collagen content, which makes interpretation of their data difficult. They assume that only a slight increase in collagen occurs and that a change in the collagen type I/III ratio may explain increased collagen-based stiffness. Measurements of the absolute content of both collagen and titin will provide data useful for the estimation of LV total stiffness. This needs to be established in future studies.

**Studies in the Human Heart**

Neagoe et al. present measurements in patients with normal hearts and in those with CAD. The tissue samples were from the midmyocardium, as in the study by Wu et al. The decrease of total muscle strip stiffness correlated with a shift toward more N2BA, a result opposite from that obtained by Wu et al. This result was confirmed in rat right ventricles with LV infarction.

Collagen-based stiffness was not determined. On the other hand, they claimed that collagen was increased on the basis of immunofluorescent histological pictures that showed a very small area of myocardium rather than on quantitative evaluation. Because studies of the myocyte structure are lacking as well, it is difficult to put this work into perspective.

**Discrepancy of Results**

It is interesting to compare the conclusions of these 2 studies. Wu et al. show a contribution to myocardial stiffness by an isofrom shift toward N2B, and Neagoe et al. propose the concept that the shift toward N2BA is an attempt to counteract the global stiffening induced by collagen/desmin accumulation. Why these 2 studies, carried out by researchers who used the same methods and who published together in previous years, present divergent results is difficult to explain. Discrepancies may have been caused by multiple factors, including appropriateness of the pacing model and of rat myocardial infarction, species differences, and heterogeneity of human pathology.
The shift to the stiffer titin isoform N2B described by Wu et al.\(^1\) may possibly be explained as a response to the sudden and significant up-regulation of heart rate by pacing. This may be similar to the situation in small rodents with a high beating frequency that show an almost exclusive N2B expression.\(^5\)

**Importance of the Present Studies and Perspectives**

The studies in the present issue by Wu et al.\(^1\) and Neagoe et al.\(^2\) are important because they show that an isoform shift of titin occurs under pathophysiological situations of cardiac overload. The authors of both studies are well-known pioneers and experts in the field of titin, having clarified most of the major issues about this interesting molecule, including complete sequencing and multiple functional properties.\(^17,18\)

We hope that the discrepancies of results will soon be reconciled, and the relationship between disturbed (Wu et al.\(^1\)) or tentatively preserved (Neagoe et al.\(^2\)) diastolic function and isoform shifts of titin will be clarified. The results could bring an improved understanding of the mechanisms underlying systolic and diastolic heart failure in human beings.

**References**


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