Commentary

Modulation of Cardiac Function: Titin Springs into Action

CHEE CHEW LIM and DOUGLAS B. SAWYER
Whitaker Cardiovascular Institute and Center for the Molecular Stress Response, Boston University School of Medicine, Boston, MA 02118

Sympathetic stimulation has become a central tenet in our understanding of how cardiac contractility is dynamically altered to accommodate the changing demands of the organism. The mechanisms by which acute and chronic sympathetic stimulation of the heart modulates cardiac output remain incompletely understood, however. Beyond the increase in heart rate driven by sympathetic stimulation of the sino-atrial node, the β-adrenergic receptor (β-AR)–mediated cascade increases contractile force development (inotropy) and accelerates relaxation (lusitropy). Numerous proteins have been discovered to be involved in the β-AR-stimulated response, including calcium handling proteins, myofilament proteins, G-proteins, and regulators of myocardial metabolism (Bers, 2002). The newest player on the field is titin (Fig. 1), the giant myofilament protein that serves as an entropic spring that imparts both the passive and the restoring forces during diastole and systole, respectively. Additional roles for titin have been proposed, including regulation of sarcomere length dependence of myofilament calcium sensitivity, a molecular template for thick filament assembly and sarcomere integrity, and centering of the A-band (Tskhovrebova and Trinick, 2003; Granzier and Labeit, 2004). Granzier and colleagues recently have demonstrated that titin is a target of PKA phosphorylation downstream of β-AR activation, resulting in a change in the titin-based passive force that increases myofilament compliance during sarcomere elongation (Yamazaki et al., 2002). In this issue, Granzier and colleagues have refined our understanding further, demonstrating that the effect of titin phosphorylation on the passive as well as the restoring force is isoform specific (Fukuda et al., 2005). This article adds to the expanding literature that demonstrates the complex role(s) of this giant protein as an important determinant of not only sarcomere structure, but also as a key regulator of dynamic changes in cardiac function over both short (beat to beat) and long (day to day) time frames.

Differential and Developmental Expression of Titin Isoforms

Full-length titin spans half the sarcomere with functionally distinct motifs in the Z-line, I-band, A-band, and M-line of the sarcomere (Tskhovrebova and Trinick, 2003; Granzier and Labeit, 2004). It is the I-band portion of titin that serves as a molecular spring that, when stretched, imparts passive and restoring forces to the cardiac myocyte, and thus influences the contractile state of the heart. The elasticity of cardiac titin is derived from three mechanically distinct, serially linked spring elements: (1) tandem immunoglobulin (Ig) repeats, (2) PEVK region (rich in proline, glutamate, valine, and lysine residues), and (3) unique N2B region (Helmes et al., 1999). Differential splicing of a single titin gene allows for length variations in the Ig repeats and PEVK segments, thus creating titins with different extensibilities (Freiburg et al., 2000). Hearts from large adult mammals (including humans) express predominantly two titin isoforms: N2B titin with a shorter extensible region and higher passive stiffness and N2BA titin with a longer extensible region and hence greater compliance (Cazorla et al., 2000). In contrast, hearts from small mammals express primarily the N2B isoform and predictably have a higher passive stiffness.

Titin isoforms also are developmentally regulated as fetal hearts express even more compliant titins, due to the insertion of additional tandem Ig repeats and PEVK sequences (fetal titins or N2BA1/N2BA2), which eventually are replaced by the adult isoforms during postnatal development (Lahmers et al., 2004; Opitz et al., 2004). In diseased hearts, a shift in coexpression of titin isoforms has been reported with a higher N2BA/N2B ratio in human dilated cardiomyopathy and a lower N2BA/N2B ratio in a pacing-induced canine heart failure model (Neagoe et al., 2002; Wu et al., 2002; Makarenko et al., 2004; Nagueh et al., 2004). Importantly, the physiological, developmental, and pathological shifts in titin isoforms that are observed in these studies appear to predict changes in myocardial stiffness and ventricular function. These results suggest that titin isoform expression is one mechanism for...
modulating passive sarcomere mechanical properties on a long time frame.

**Acute Regulation of Titin Mechanics**

In addition to PKA phosphorylation of titin, Granzier and colleagues have reported a separate mechanism for rapid adjustment of the molecular spring constant of titin that also is isoform specific. In single molecules, calcium lowers the bending rigidity of the PEVK segments that contain E-rich motifs (Labeit et al., 2003), which is present in N2BA but not in N2B titin. Accordingly, skinned muscle fibers with predominantly N2BA showed a calcium-dependent increase in titin passive force, which was absent in fibers expressing primarily N2B, and the calcium-sensitive stiffness of the more compliant N2BA titin was postulated to stabilize the sarcomeres during contraction (Fujita et al., 2004). Interestingly, previous work by the same group and others (Kulke et al., 2001; Yamasaki et al., 2001) revealed that the PEVK segment of N2B titin interacts with actin, in vitro, and that this interaction is inhibited in the presence of calcium by the calcium-binding protein S100A1 (Yamasaki et al., 2001). Whether titin-based tension is dynamically modulated during a single cardiac cycle by calcium, particularly in vivo, remains to be determined.

Hearts expressing predominantly N2B titin (such as small rodents) are stiffer and have a higher intrinsic heart rate, with reduced diastolic filling times. Yamasaki et al. (2002) proposed that a reduction in titin passive tension via PKA phosphorylation would allow for a more rapid and complete ventricular filling, thereby increasing end-diastolic volume and cardiac output. This interpretation, however, does not take into account a possible effect of calcium/S100A1 on titin passive tension. In the article by Fukuda et al. (2005), the PKA-induced changes are diminished in intact muscle fibers from rat ventricle (Fig. 7, where S100A1 is present) when compared with skinned fibers (Fig. 2, where calcium is kept constant). Perhaps this is due to an offset of the PKA effect by an increase in passive tension via PEVK–actin interactions, as the inhibitory effect of S100A1 diminishes with the fall in calcium during diastole.

Fukuda et al. (2005) also demonstrate that the reduction in titin tension by PKA phosphorylation lowers restoring force at short sarcomere lengths. It is interesting to speculate about the effect of this on cardiac output. Fukuda and colleagues suggest that lowering the restoring force might have detrimental effects on myocardial efficiency due to a reduction of the length-dependent deactivation effect of titin at the systolic–diastolic transition. One could also argue that a reduction in restoring force will improve cardiac output. The drop in restoring force would predictably allow for lower end-systolic volumes at any given afterload by decreasing the resistance to myofilament shortening, allowing the ventricle to reach lower chamber volumes, and thus increasing stroke volume. In addition, the decrease in titin-based tension due to PKA phosphorylation is further enhanced during systole by inhibition of PEVK–actin interaction via S100A1 as calcium levels rise. Thus, the PKA and calcium effects on titin-based restoring force work together to enhance ventricular emptying and increase cardiac output. The overall im-

---

**Figure 1.** Excitation–contraction (EC) coupling and the response to β-adrenergic receptor (β-AR) stimulation. (A) EC coupling involves depolarization of the transverse tubule that activates voltage-gated L-type calcium channels (LTCC). Influx of calcium through LTCC triggers a greater calcium release from the SR into the cytoplasm via ryanodine receptor (RyR) channels, which activates contraction. Relaxation occurs when cytoplasmic calcium is resequestered by the SR calcium-ATPase (SERCA2a), which is regulated by phospholamban (PLB). The excess calcium that entered the cell via the LTCCs is eventually extruded by the sarcolemmal sodium/calcium exchanger (NCX). (B) β-AR stimulation involves binding of epinephrine and norepinephrine to the receptor, G protein–mediated activation of adenylate cyclase (AC), synthesis of cyclic AMP (cAMP), and activation of PKA. PKA-dependent phosphorylation of calcium handling and myofilament proteins are depicted in red. Asterisk denotes potential modulation of titin spring constant by calcium and/or calcium/S100A1. The overall effect of PKA phosphorylation is an augmentation in myocardial inotropy and lusitropy.
Impact of a diminished titin-based restoring force on the efficiency of the systolic–diastolic transition may be minimized as elastic recoil is partially restored with the reversal of S100A1 inhibition on PEVK–actin interaction as calcium levels fall during diastole. In hearts expressing both N2B and N2BA isoforms, the picture becomes even more complex. In addition to the isoform-specific effects of PKA, the N2BA and N2B effects of calcium and calcium/S100A1 (which have opposing actions on titin stiffness) also must be taken into consideration.

While mostly speculative at this point, the ability of PKA and calcium to dynamically vary titin stiffness within a single cardiac cycle is a new and intriguing concept. Further studies are warranted to fully elucidate the extent to which these events independently or in concert regulate cardiac function.

Isoform Shifts in Diseased Hearts: Adaptive or Maladaptive?

One cannot help but marvel at how the cardiac myocyte has evolved this ability to dynamically regulate length-dependent changes in cardiac contractility, both on a short timescale through phosphorylation–dephosphorylation and calcium regulation of titin stiffness, and on a longer time scale through changes in isoform expression. This adaptability arguably maximizes myocardial efficiency, by changing the spring constant and myofilament calcium-sensitizing properties of titin to match the workload. It is interesting that the short-term changes in titin mechanics appear to be most tightly coupled to the inotropic state of the heart. In contrast, the long-term changes in isoform expression appear to relate most clearly to ventricular chamber size and/or resting heart rate.

Based upon Granzier and colleagues’ prior work, though, there appears to be a maladaptive loss of the ability of the myofilaments to dynamically regulate passive tension in the setting of chronic heart failure. Naghedi et al. (2004) reported recently that in the ventricle of patients with end-stage human heart failure there is an increase in the N2BA/N2B ratio. A careful evaluation of the mechanical properties of the myocardium revealed that the shift toward increased expression of the longer isoform led to an increase in the titin-based compliance of the cardiac muscle. Moreover, the investigators were able to show that the in vivo parameters of diastolic function at rest correlated with the N2BA/N2B ratio. Thus at rest, they proposed that a shift toward the longer N2BA isoform might be considered an adaptive strategy to improve cardiac diastolic function in the failing heart (LeWinter, 2004). However like many adaptations to the pathological condition of reduced cardiac output, this appears to be at least “short-sighted,” if not maladaptive. The longer N2BA isoform will maximize compliance of the ventricle, and allow the heart to begin systole at longer sarcomere lengths, and higher end-diastolic volumes. With an impaired Frank-Starling mechanism for length-dependent activation, the expected gain in contractility normally associated with sarcomere elongation will be absent. Given the new finding that the N2BA isoform does not change stiffness in response to PKA-dependent phosphorylation provides another mechanism for the impaired responsiveness of the failing heart to β-AR stimulation. Moreover, the higher end-diastolic volumes that will be achieved with the less stiff N2B isoform will result in an increase in wall stress at any given pressure, and therefore may add in a feed-forward manner to the well-characterized process of ventricular remodeling. Further work is needed to understand the obviously complex mechanisms regulating titin isoform expression, and the extent to which these changes are desirable or undesirable adaptations to conditions of increased load.

We are supported by American Heart Association Scientist Development grant 0430087N (C.C. Lim) and National Institutes of Health grant HL-68144 (D.B. Sawyer).

REFERENCES

Bers, D.M. 2002. Cardiac excitation-contraction coupling. Nature. 415:198–205.

Cazorla, O., A. Freiburg, M. Helmes, T. Centner, M. McNabb, Y. Wu, K. Trombitas, S. Labeit, and H. Granzier. 2000. Differential expression of cardiac titin isoforms and modulation of cellular stiffness. Circ. Res. 86:59–67.

Freiburg, A., K. Trombitas, W. Hell, O. Cazorla, F. Fougerousse, T. Centner, B. Kolmerer, G. Witt, J.S. Beckmann, C.C. Gregorio, et al. 2000. Series of exon-skipping events in the elastic spring region of titin as the structural basis for myofibrillar elastic diversity. Circ. Res. 86:1114–1121.

Fujita, H., D. Labeit, B. Gerull, S. Labeit, and H.L. Granzier. 2004. Titin isoform-dependent effect of calcium on passive myocardial tension. Am. J. Physiol. Heart Circ. Physiol. 287:H2528–H2534.

Fukuda, N., P. Nair, Y. Wu, and H. Granzier. 2005. Phosphorylation of titin modulates passive stiffness cardiac muscle in a titin isoform–dependent manner. J. Gen. Physiol. 125:257–271.

Granzier, H.L., and S. Labeit. 2004. The giant protein titin: a major player in myocardial mechanics, signaling, and disease. Circ. Res. 94:284–295.

Helmes, M., K. Trombitas, T. Centner, M. Kellermayer, S. Labeit, W.A. Linke, and H. Granzier. 1999. Mechanically driven contour-length adjustment in rat cardiac titin’s unique N2B sequence: titin is an adjustable spring. Circ. Res. 84:1339–1352.

Kulke, M., S. Fujita-Becker, E. Rostkova, C. Neagoe, C. Labeit, D.J. Manstein, M. Gautel, and W.A. Linke. 2001. Interaction between PEVK-titin and actin filaments: origin of a viscous force component in cardiac myofibrils. Circ. Res. 89:874–881.

Labeit, D., K. Watanabe, C. Witt, H. Fujita, Y. Wu, S. Lahmers, T. Funck, S. Labeit, and H. Granzier. 2005. Calcium-dependent molecular spring elements in the giant protein titin. Proc. Natl. Acad. Sci. USA. 102:13716–13721.

Lahmers, S., Y. Wu, D.R. Call, S. Labeit, and H. Granzier. 2004. Developmental control of titin isoform expression and passive stiffness in fetal and neonatal myocardium. Circ. Res. 94:505–513.

LeWinter, M.M. 2004. Titin isoforms in heart failure: are there ben-
fits to supersizing? *Circulation.* 110:109–111.
Makarenko, I., C.A. Opitz, M.C. Leake, C. Neagoe, M. Kulke, J.K. Gwathmey, F. del Monte, R.J. Hajjar, and W.A. Linke. 2004. Passive stiffness changes caused by upregulation of compliant titin isoforms in human dilated cardiomyopathy hearts. *Circ. Res.* 95: 708–716.
Nagueh, S.F., G. Shah, Y. Wu, G. Torre-Amione, N.M. King, S. Lahmers, C.C. Witt, K. Becker, S. Labeit, and H.L. Granzier. 2004. Altered titin expression, myocardial stiffness, and left ventricular function in patients with dilated cardiomyopathy. *Circulation.* 110:155–162.
Neagoe, C., M. Kulke, F. del Monte, J.K. Gwathmey, P.P. de Tombe, R.J. Hajjar, and W.A. Linke. 2002. Titin isoform switch in ischemic human heart disease. *Circulation.* 106:1333–1341.
Opitz, C.A., M.C. Leake, I. Makarenko, V. Benes, and W.A. Linke. 2004. Developmentally regulated switching of titin size alters myofibrillar stiffness in the perinatal heart. *Circ. Res.* 94:967–975.
Tskhovrebova, L., and J. Trinick. 2003. Titin: properties and family relationships. *Nat. Rev. Mol. Cell Biol.* 4:679–689.
Wu, Y., S.P. Bell, K. Trombitas, C.C. Witt, S. Labeit, M.M. LeWinter, and H. Granzier. 2002. Changes in titin isoform expression in pacing-induced cardiac failure give rise to increased passive muscle stiffness. *Circulation.* 106:1384–1389.
Yamasaki, R., M. Berri, Y. Wu, K. Trombitas, M. McNabb, M.S. Kellermayer, C. Witt, D. Labeit, S. Labeit, M. Greaser, and H. Granzier. 2001. Titin-actin interaction in mouse myocardium: passive tension modulation and its regulation by calcium/S100A1. *Biophys. J.* 81:2297–2313.
Yamasaki, R., Y. Wu, M. McNabb, M. Greaser, S. Labeit, and H. Granzier. 2002. Protein kinase A phosphorylates titin’s cardiac-specific N2B domain and reduces passive tension in rat cardiac myocytes. *Circ. Res.* 90:1181–1188.