Regulation of Myocardial Contractility by a Downstream Mechanism

Masao Endoh

Binding of intracellular Ca\textsuperscript{2+} ions to troponin C subsequent to membrane excitation triggers the interaction of actin with myosin molecules by displacing the inhibition induced by troponin I at diastolic levels of [Ca\textsuperscript{2+}]. Therefore, in intact myocardial cells, the amplitude and rate of tension development and relaxation are primarily determined by the rate of Ca\textsuperscript{2+} mobilization and deprivation, by the crossbridge cycling rate, or by the contribution of both. From this perspective, there are 3 general types of mechanisms by which it should be possible to alter the contractile performance of cardiac muscle. Binding of Ca\textsuperscript{2+} to troponin C plays a key role and is considered to be the central mechanism of cardiac excitation-contraction coupling. The regulation of the Ca\textsuperscript{2+} mobilizing process is regarded as the upstream mechanism; the process subsequent to Ca\textsuperscript{2+} binding to troponin C (ie, an alteration of the response of the myofilaments to a given level of occupancy of Ca\textsuperscript{2+} binding sites on troponin C) is regarded as the downstream mechanism.\textsuperscript{1}

The mechanistic analysis of the role of Ca\textsuperscript{2+} ions in the cardiac contractile regulation in intact myocardial cells has progressed significantly since the introduction of methods to apply the Ca\textsuperscript{2+}-sensitive photoprotein (aequorin) and fluorescent dyes (eg, fura-2, indo-1, and fluo-3) in intact myocardial cells.\textsuperscript{2–4} The majority of inotropic interventions alter the intracellular Ca\textsuperscript{2+} transient. The increase in frequency of contraction (force-frequency relationship) and cardiotoxic agents, such as \beta-adrenoceptor agonists, digitalis, and phosphodiesterase III inhibitors, act primarily through the upstream mechanism. By contrast, length-dependent regulation (Frank-Starling mechanism), activation of receptors coupled to the stimulation of phosphoinositide hydrolysis (\alpha-adrenoceptors, endothelin, and angiotensin AT\textsubscript{1} receptors), and actions of novel Ca\textsuperscript{2+} sensitizers (eg, EMD 57033 and Org 30029) are associated with a relatively small alteration or no alteration in Ca\textsuperscript{2+} transients. These are postulated to act through the central and/or downstream mechanism. The upstream mechanism can be easily distinguished from the other 2 mechanisms by examination of the relationship between the amplitude of the Ca\textsuperscript{2+} transient and the force developed in twitch contraction or by an analysis of the steady-state tension-pCa relationship during application of tetanic stimulation in the presence of a dysfunction of the cardiac sarcoplasmic reticular Ca\textsuperscript{2+} pump induced by ryanodine or thapsigargin in intact myocardial cells loaded with Ca\textsuperscript{2+} indicators.\textsuperscript{3,4} Application of these experimental procedures to analysis of the downstream mechanism, however, is difficult and limited. It is evident that the downstream mechanism includes 2 processes: (1) the interaction of the troponin-tropomyosin complex with actin and (2) crossbridge cycling itself. The combination of the in vitro motility assay, in which the movement of visualized thin filaments or actin molecules over a fixed layer of myosin can be directly investigated, the \textsuperscript{45}Ca\textsuperscript{2+}-binding assay, and the force-pCa relationship in cardiac skinned fibers differentiates the 2 processes. The first process shifts the force-pCa curve to the left in skinned fibers but does not increase the amount of \textsuperscript{45}Ca\textsuperscript{2+} binding to troponin C and requires the troponin-tropomyosin complex for regulation in the in vitro motility assay, whereas the second process does not require the complex.\textsuperscript{3,4}

In this issue of Circulation Research, Palmer and Kentish\textsuperscript{5} have elegantly characterized the crossbridge cycling rate after isolation of the downstream mechanism from the central mechanism by application of caged compounds to skinned cardiac fibers isolated from rat and guinea pig ventricular trabeculae. The force-pCa relationships for both species were sigmoidal, fitted by the Hill equation, and superimposable, an indication that the myofibrillar Ca\textsuperscript{2+} sensitivity (Ca\textsuperscript{2+} binding affinity of troponin C) in the 2 species was the same. Since the Ca\textsuperscript{2+} binding sites of troponin C could be abruptly saturated by Ca\textsuperscript{2+} released from a caged Ca\textsuperscript{2+} compound, NP-EGTA, by photolysis with application of flash, the maximal rate of crossbridge cycling could be elicited in skinned fibers from the rat and guinea pig under the same experimental conditions. The former process of the downstream mechanism, ie, modulation at the level of interaction of the troponin-tropomyosin complex with actin could also be bypassed in the experiment of Palmer and Kentish\textsuperscript{5} because no interventions that might have potential action on this process were applied in their study. The rate constant of Ca\textsuperscript{2+} activation of force (k\textsubscript{act}) at the maximum flash energy in rat trabeculae was 5-fold greater than that in guinea pig trabeculae, indicating that rat trabeculae were activated 5-fold faster than were trabeculae from guinea pigs. Interestingly and importantly, the rate constant for force redevelopment (k\textsubscript{v}) after forcible detachment of the crossbridges determined during maximal activation (pCa 4.5) in the rat was also 4.5-fold greater than that in the guinea pig, and these values were not significantly different from the maximum values of k\textsubscript{act} measured in the respective species. This indicates that the different k\textsubscript{act} values in the 2 species apparently reflect the relative rates of the force-generating processes. It is important to note that k\textsubscript{act} was measured after sudden alteration of [Ca\textsuperscript{2+}], whereas k\textsubscript{v}, which reflects the rate of crossbridge

\textsuperscript{1}The opinions expressed in this article are not necessarily those of the editor or of the American Heart Association.

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reattachment and transition to force-generating states, was determined under constant activation by Ca\(^{2+}\), thus bypassing any direct influence of [Ca\(^{2+}\)] on the rate at which Ca\(^{2+}\) ions trigger conformational changes in the regulatory proteins of the thin filament. The consistency of these 2 values provides strong support for the correctness of the experimental procedure to produce an immediate elevation of [Ca\(^{2+}\)], by means of photolysis of a caged Ca\(^{2+}\) compound in skinned cardiac fibers. Under such conditions, the authors have clearly demonstrated that the maximum rate of Ca\(^{2+}\)-activated force development is limited by the rate at which detached or weakly bound crossbridges enter the force-generating state (downstream mechanism) rather than by the rate at which the thin filament is switched on by Ca\(^{2+}\) binding to troponin C (central mechanism). It has been known that the distribution of myosin isozymes involves a wide range of variation among mammalian species. The rat ventricle contains mostly V\(_1\) myosin (αα myosin heavy chains), whereas V\(_2\) and V\(_3\) myosins (αβ and ββ heavy chains) predominate in the guinea pig. In various biochemical and mechanical assays, crossbridges containing V\(_1\) myosin have a 2- to 6-fold higher cycling rate compared with those containing V\(_3\) myosin, which may explain, in large part, the faster activation rates in the rat compared with the guinea pig. Since the human ventricle contains mostly V\(_1\) myosins, analysis of the similarity and dissimilarity by comparison with these species by means of the methods of Palmer and Kentish\(^5\) may shed light on the regulation in human ventricular muscle. In this context, it is also noteworthy that the comparison of unitary displacements and forces between V\(_1\) and V\(_3\) myosin by means of optical trap techniques in the in vitro motility assay revealed that both the unitary displacements and forces of the 2 myosin isofoms are similar in amplitude but different in duration.\(^6\)

The above consideration also accounts for the difference in the rate of relaxation between the rat and the guinea pig. The \(k_{\text{act}}\) values in the 2 species were not significantly different from the corresponding maximum rates of relaxation determined by a sudden drop of solution [Ca\(^{2+}\)] by use of flash photolysis of diazo-2, a caged chelator of Ca\(^{2+}\). The rate of myocardial relaxation may be determined either by the rate at which Ca\(^{2+}\) dissociates from troponin C (\(k_{\text{act}}\)) and causes thin filament deactivation (central mechanism) or by the net rate at which myosin crossbridges detach from actin once Ca\(^{2+}\) ions are lost from troponin C (downstream mechanism). The similarity of the force-pCa relationships in the rat and guinea pig implies that \(k_{\text{act}}\) should be equivalent in both species. Thus, the 5-fold difference in relaxation rate between the 2 species suggests that the relaxation rate caused by photolysis of diazo-2 may reflect the detachment rate of crossbridges themselves but not \(k_{\text{off}}\), which may be due to the difference between the myosin isoform composition in the 2 species.\(^5\)

Palmer and Kentish\(^5\) are the first to carry out a careful comparison of the rate of relaxation with \(k_{\text{act}}\) or \(k_{\text{p}}\) under the same experimental conditions and in the same animal species. The rate of relaxation was similar to \(k_{\text{act}}\) or \(k_{\text{p}}\) in a given species. These findings do not fit the crossbridge cycling model for skeletal muscle proposed previously\(^7\): in the kinetic model, the rate of relaxation, which occurs at lower [Ca\(^{2+}\)], than activation, should be lower than \(k_{\text{act}}\) or \(k_{\text{p}}\) (see Palmer and Kentish\(^5\) for detailed discussion). The authors, therefore, postulated that during relaxation crossbridges may subsequently lose force by reversal of the force-generating transition (crossbridge working stroke) rather than by the slower forward detachment step.\(^5\) The fact that \(k_{\text{act}}\) is consistent with \(k_{\text{p}}\) provides strong support for the conclusion that the \(k_{\text{act}}\) determined by the photolysis may reflect accurately the true rate of activation of crossbridges under the experimental conditions. In addition, the mode of dependence of \(k_{\text{act}}\) and \(k_{\text{p}}\) on the extent of contractile activation corresponds with the activation dependence of the crossbridge cycling rate in the current concept of cardiac excitation-contraction coupling. On the other hand, the rate of relaxation solely determined by means of photolysis of the caged Ca\(^{2+}\)-chelating compound diazo-2 may likely involve yet-unknown factors that contribute to the overestimation of the rate of relaxation in skinned cardiac fibers and also to the difference in relaxation kinetics between the rat (double exponential) and the guinea pig (single exponential).\(^5\) Simnett and coworkers\(^8-10\) have reported an even lower rate constant of activation (7.15 s\(^{-1}\)) than of relaxation (15.4 s\(^{-1}\)), determined by photolysis of nitr-5 and diazo-2, respectively, at 12°C. Further study focusing on the factors contributing to the rate of relaxation, including the development of this new experimental model in cardiac muscle as proposed by the authors,\(^5\) should be pursued.

Increasing interest has been focused on the elucidation of the basis of the downstream mechanism and its regulation by cardiotonic agents, because the downstream mechanism may bypass the risk of Ca\(^{2+}\) overload that leads to arrhythmias, myocardial cell injury, and energetic disadvantages that are associated with cardiotonic agents acting through the upstream mechanism. Furthermore, they may be effective in regulating force even under pathophysiological conditions such as acidosis and myocardial stunning. It has already been demonstrated that the actions of Ca\(^{2+}\) sensitizers acting primarily on the downstream mechanism reveal different characteristics among the compounds in respect to regulation of the rate of activation and detachment of crossbridges. EMD 57033 increased preferentially the rate of activation and accelerated slightly the rate of relaxation in skinned guinea pig cardiac trabeculae.\(^8,9\) In the rat cardiac skinned fibers loaded with diazo-2, CGP 48506 did not affect the rate of relaxation, whereas caffeine accelerated the relaxation rate.\(^10\) A potential and serious disadvantage accompanied by the action of Ca\(^{2+}\) sensitizers is an impairment of relaxation that may lead to diastolic dysfunction in the clinical setting. In contrast to EMD 57033, levosimendan did not impair the relaxation in intact myocardial cells.\(^11\) In this respect, the experimental procedure to produce a rapid alteration of [Ca\(^{2+}\)] by means of the photolysis of caged compounds in skinned cardiac fibers is important for elucidation of the mechanism of action of Ca\(^{2+}\) sensitizers, which may provide useful information for the clinical application of these agents as well as for analysis of basic molecular mechanisms of cardiac muscle contraction.

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