Effects of MgADP on Length Dependence of Tension Generation in Skinned Rat Cardiac Muscle

Norio Fukuda, Hidetoshi Kajiwara, Shin’ichi Ishiwata, Satoshi Kurihara

Abstract—The effect of MgADP on the sarcomere length (SL) dependence of tension generation was investigated using skinned rat ventricular trabeculae. Increasing SL from 1.9 to 2.3 μm decreased the muscle width by ∼11% and shifted the midpoint of the pCa-tension relationship (pCa_{50}) leftward by about 0.2 pCa units. MgADP (0.1, 1, and 5 mmol/L) augmented maximal and submaximal Ca^{2+}-activated tension and concomitantly diminished the SL-dependent shift of pCa_{50} in a concentration-dependent manner. In contrast, pimobendan, a Ca^{2+} sensitizer, which promotes Ca^{2+} binding to troponin C (TnC), exhibited no effect on the SL-dependent shift of pCa_{50}, suggesting that TnC does not participate in the modulation of SL-dependent tension generation by MgADP. At a SL of 1.9 μm, osmotic compression, produced by 5% wt/vol dextran (molecular weight ≈464 000), reduced the muscle width by ∼13% and shifted pCa_{50} leftward to a similar degree as that observed when increasing SL to 2.3 μm. This favors the idea that a decrease in the interfilament lattice spacing is the primary mechanism for SL-dependent tension generation. MgADP (5 mmol/L) markedly attenuated the dextran-induced shift of pCa_{50}, and the degree of attenuation was similar to that observed in a study of varying SL. The actomyosin-ADP complex (AM.ADP) induced by exogenous MgADP has been reported to cooperatively promote myosin attachment to the thin filament. We hereby conclude that the increase in the number of force-generating crossbridges on a decrease in the lattice spacing is masked by the cooperative effect of AM.ADP, resulting in depressed SL-dependent tension generation. The full text of this article is available at http://www.circresaha.org. (Circ Res. 2000;86:e1-e6.)

Key Words: MgADP • pimobendan • Ca^{2+} sensitivity • cardiac muscle • sarcomere length

An alteration in ventricular end-diastolic volume results in a marked change in cardiac output. This intrinsic ability of the heart to alter cardiac output forms the basis for the Frank-Starling law of the heart. It is well established that twitch tension and Ca^{2+} responsiveness in cardiac muscle preparations are enhanced as muscle length (ie, sarcomere length [SL]) is increased within the normal physiological range (SL from ∼1.8 to ∼2.3 μm). Although a number of studies have been conducted to account for the SL dependence of tension generation in living myocardium, its mechanism has not been completely elucidated. However, at the myofilament level, there is an increasing amount of evidence suggesting that the SL dependence is primarily due to a change in the interfilament lattice spacing that accompanies the SL change. A possible consequence of the decreased lattice spacing is an increase in the probability of myosin attachment to the thin filament, resulting in an increase in the number of force-generating crossbridges. Ishiwata and Oosawa proposed a model based on the Ca^{2+}-dependent flexibility of the thin filament, in which they assumed that (1) the muscle volume (ie, the lattice volume) remains constant on a change in SL and that (2) there is a critical distance between the thick and thin filaments for tension generation. This model quantitatively explains both the stretch-induced increase in the steady isometric tension and the slower (or faster) rate of tension development (or decline) at a shorter SL. Supporting the hypothesis that a change in the lattice spacing plays a pivotal role in determining the SL-dependent Ca^{2+} sensitivity of tension. Alternatively, it was proposed that the length-dependent change in myofilament activation is caused by cardiac troponin C (TnC), which acts as a "length sensor" in the cardiac muscle contractile system. However, this idea has attained little experimental evidence from other groups, and it was challenged by McDonald et al, who reported that the expression of skeletal TnC in ventricular myocytes of transgenic mice did not alter the SL dependence of Ca^{2+} sensitivity of tension in skinned myocytes. Thus, it is unlikely that TnC alone acts as a "length sensor" in cardiac muscle. It is known that the degree of activation of the thin filament is regulated not only by the binding of Ca^{2+} to TnC but also...
by the formation of strong-binding crossbridges, such as the rigor18,19 and crossbridges that bind ADP.20–22 Fukuda et al22 reported that the actomyosin-ADP complex (AM-ADP) induced by exogenous MgADP can “turn on” adjacent actin molecules in a cooperative manner so that the actin and myosin interaction becomes possible, just as if Ca2+ were bound to TnC. Consequently, upon the addition of MgADP, the pCa-tension relationship for skinned cardiac muscle is shifted to the left, showing greater Ca2+ sensitivity of tension.21–23

To investigate the influence of the formation of strong-binding crossbridges on SL-dependent tension generation in cardiac muscle, we measured the SL-dependent shift of the pCa-tension relationship in the presence of varying concentrations of MgADP using skinned rat ventricular trabeculae. The formation of strong-binding crossbridges is known to increase the affinity of TnC for Ca2+.5,24 Thus, to clarify whether the effect of MgADP is based on the increased affinity of TnC for Ca2+, we examined the effect of pimobendan, a Ca2+ sensitizer, which promotes Ca2+ binding to TnC.25,26 A preliminary report has been published in abstract form.27

Materials and Methods

Experimental Procedure

The heart was removed from male Wistar rats (250 to 300 g) anesthetized with sodium pentobarbital (50 mg/kg IP). The rats were supplied by Saitama Experimental Animals Supply (Saitama, Japan), and the present study conforms with the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan. Thin trabecula muscles with the diameter of 100 to 150 μm were dissected from the right ventricle in oxygenated Tyrode solution without Ca2+ at 30°C. The preparations were skinned by superfusion with 1% vol/vol Triton X-100 in the relaxing solution (in mmol/L: MgATP 4, MOPS 10, EGTA 10, free Mg2+ 1, and ionic strength 180 [pH 7.0]) for 60 minutes at ~2°C. The ionic strength (IS) was adjusted with KCl. The preparations were then washed with the relaxing solution to remove Triton X-100 and stored at ~20°C in the relaxing solution containing 50% vol/vol glycerol and 2 mmol/L leupeptin for 1 week or less.

Both ends of the preparation were tied to thin tungsten wires with a silk thread. One end was attached to a tension transducer (BG-10; Kulite Semiconductor Products, Inc, Leonia, NJ) and the other to a micromanipulator (Narishige, Tokyo, Japan). The SL was adjusted to either 1.9 or 2.3 μm by measuring laser light diffraction in the relaxing solution. Ca2+-activated isometric tension was measured in activating solutions containing 4 mmol/L MgATP, 10 mmol/L MOPS, 1 mmol/L free Mg2+, a varying concentration of free Ca2+ (adjusted with Ca[10 mmol/L EGTA]), 0.1 mmol/L P, P-diadenosine-5 ’-pentaphosphate (AP5A), 15 mmol/L creatine phosphate (CP), 15 U/ml creatine phosphokinase (CPK), and 180 mmol/L IS [pH 7.0], at the two SLs with/without MgADP or pimobendan (donated by Nippon Boehringer Ingelheim; Kawanishi, Hyogo, Japan).

The control pCa-tension relationship without MgADP or pimobendan was first obtained at a SL of 1.9 μm and then at 2.3 μm. By using the same preparation, the pCa-tension relationships in the presence of MgADP or pimobendan were obtained at the two SLs. Each pCa-tension relationship was obtained by cumulatively raising the Ca2+ concentration from the relaxing condition. Because we noted a variation in nH (and related parameters), depending on the preparation, paired experiments were carried out on the same preparation. Finally, maximal Ca2+-activated tension (at pCa 4.8) was measured at the two SLs in the control condition without MgADP or pimobendan to examine the reproducibility of tension development. We only used the data in which the final tension values were greater than 70% of those measured at the beginning of the experiment.

The muscle width was measured under a microscope (Nikon SMZ645) at a magnification of ×225. The concentrations of chemicals in solutions were estimated by computer calculation.28 All experiments were carried out at 20±0.2°C.

Data and Statistical Analyses

The pCa-tension relationship was fitted to the Hill equation: [log[P/(100−P)]=nH[pCa0−pCa], where P is the relative tension expressed as a percentage of the maximum (Ca2+, pCa 4.8), nH is the Hill coefficient, and pCa0 is −log(Ca2+) at P=0. All data are expressed as mean±SEM. Paired Student’s t test was used, and statistical significance was verified at P<0.05.

Results

Effect of MgADP on the Length Dependence of Ca2+ Sensitivity of Tension

Figure 1 shows the effect of MgADP on the SL dependence of Ca2+ sensitivity of tension. In the control condition without MgADP or pimobendan, maximal absolute Ca2+-activated tension values were 52.5±4.7 and 77.9±4.1 mg (n=17; P<0.001) at SL 1.9 and 2.3 μm, respectively, and pCa0 was shifted leftward by about 0.2 pCa units by increasing SL from 1.9 to 2.3 μm. The degree of the SL-dependent shift of pCa0 was consistent with the result of a previous study using rat ventricular muscle strips.29 In the absence of MgADP, the muscle width was reduced from 132±6 to 118±4 μm (P<0.001) on extension of SL during relaxation (ie, ~11% reduction): The addition of MgADP (up to 5 mmol/L) did not change the muscle width at either SL (131±7 and 117±5 μm [n=4; P<0.001] at SL 1.9 and 2.3 μm, respectively, in the presence of 5 mmol/L MgADP).

Consistent with our previous studies using skinned bovine cardiac muscle,22,23 MgADP shifted the pCa-tension relationship to the left in a concentration-dependent manner (Figure 1). Concomitantly, the SL-dependent shift of the pCa-tension relationship was diminished in a concentration-dependent manner; in the presence of 5 mmol/L MgADP, ΔpCa0 was decreased to ~20% of the control value (Figure 1C, inset).

Change in Hill Coefficient With MgADP

<table>
<thead>
<tr>
<th>SL</th>
<th>Control</th>
<th>0.1 mmol/L</th>
<th>Control</th>
<th>1 mmol/L</th>
<th>Control</th>
<th>5 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 μm</td>
<td>5.81±0.28</td>
<td>5.78±0.40</td>
<td>4.77±0.45</td>
<td>4.74±0.32</td>
<td>4.96±0.55</td>
<td>2.43±0.08*</td>
</tr>
<tr>
<td>2.3 μm</td>
<td>5.10±0.29</td>
<td>5.03±0.56</td>
<td>4.17±0.14</td>
<td>3.97±0.07</td>
<td>5.31±0.31</td>
<td>2.21±0.10*</td>
</tr>
</tbody>
</table>

Values from left and right columns were obtained from Figures 1A, 1B, and 1C, respectively (mean±SEM [n=4 to 5]). *P<0.05 compared with corresponding control (−MgADP) values.
The Table summarizes the nH values of the pCa-tension relationships shown in Figure 1. MgADP at a concentration of 5 mmol/L significantly decreased nH at both SLs, whereas no significant changes were observed for 0.1 and 1 mmol/L MgADP. Because the ATP regenerating system (CP-CPK) was not used in the presence of MgADP, we estimated the concentration of contaminating MgADP inside the preparation. In the absence of MgADP, the pCa50 values obtained with and without CP-CPK were 5.45 ± 0.05 and 5.53 ± 0.05, respectively (n = 4; P < 0.001), at SL 1.9 μm. Thus, we estimated the contaminant MgADP to be ≈0.1 mmol/L under our experimental condition (see Figure 1A). This estimation is within the range of reported values for skinned cardiac and skeletal muscles.

Effect of Pimobendan on the Length Dependence of Ca2+ Sensitivity of Tension

Pimobendan was reported to shift the pCa-tension relationship to the left with little influence on maximal Ca2+-activated tension in skinned porcine ventricular muscle. In the present study, 10−4 mol/L pimobendan substantially shifted the pCa-tension curve to the left at SLs of 1.9 and 2.3 μm, whereas in contrast to MgADP, pimobendan did not diminish the SL-dependent shift of the pCa-tension relationship (Figure 2). The nH values in the absence and presence of pimobendan were 4.01 ± 0.27 and 3.93 ± 0.15 (n = 4; P > 0.1), respectively, at SL 1.9 μm and 4.45 ± 0.32 and 3.75 ± 0.12 (n = 4; P > 0.1), respectively, at SL 2.3 μm. Pimobendan did not significantly change nH at either SL.

Effect of MgADP or Pimobendan on Maximal Tension

Figure 3 summarizes the effect of MgADP or pimobendan on maximal Ca2+-activated tension (pCa 4.8) at SLs of 1.9 and 2.3 μm. It has been reported that MgADP significantly potentiates maximal Ca2+-activated tension in cardiac22,23,33 and skeletal muscles.20,33 We also found that MgADP augmented maximal tension in a concentration-dependent manner, and this potentiating effect was significantly less pro-
Figure 3. Effects of MgADP or pimobendan on maximal Ca\(^{2+}\)-activated tension. Conditions are the same as in Figures 1 and 2 for MgADP and pimobendan, respectively, except that pCa was fixed at 4.8. Open columns show SL 1.9 \(\mu\)m, and filled columns show SL 2.3 \(\mu\)m. NS indicates not significant. The degree of increase in tension was determined with respect to the maximal tension obtained without MgADP or pimobendan at each SL. Vertical bars indicate SEM of 4 to 5 data points for MgADP and 4 data points for pimobendan.

nounced at the longer SL (\(P<0.01\)). In contrast, pimobendan only minimally increased maximal tension at both SLs, and there was no significant difference in the increases in maximal tension between the two SLs.

Effect of MgADP on Dextran-Induced Shift of the pCa-Tension Relationship

Dextran (5\% wt/vol) reduced the width of muscle from 135±5 to 117±4 \(\mu\)m (n=7; \(P<0.001\)) under the relaxing condition at SL 1.9 \(\mu\)m. The degree of reduction (≈13\%) was similar to that observed when increasing SL to 2.3 \(\mu\)m without dextran (≈11\%, see above).

In the absence of MgADP, dextran shifted pCa\(_{50}\) to the left by 0.19±0.03 pCa units (Figure 4). The degree of the shift of pCa\(_{50}\) was comparable, albeit slightly smaller, to what was observed when increasing SL to 2.3 \(\mu\)m (see Figures 1 and 2).

Maximal tension was also augmented by ≈15\% in the presence of dextran as previously reported by other investigators using skinned skeletal11 and cardiac muscles.34

It was found that in the presence of 5 mmol/L MgADP, the dextran-induced increase in apparent Ca\(^{2+}\) sensitivity was markedly diminished (Figure 4), and the degree of attenuation was similar to what was obtained in a study of varying SL (see Figure 1C).

The nH values in the absence and presence of 5 mmol/L MgADP were 6.32±0.61 and 2.52±0.18 (n=7; \(P<0.05\)), respectively, without dextran and 5.29±0.31 and 1.68±0.06 (n=7; \(P<0.05\)), respectively, with dextran.

Discussion

We demonstrated that using skinned rat ventricular trabeculae, MgADP increases the Ca\(^{2+}\) sensitivity of tension and reduces the magnitude of SL-dependent changes in Ca\(^{2+}\) sensitivity. On the other hand, a simple increase in the Ca\(^{2+}\) binding affinity of TnC by pimobendan had no effect on the SL-dependent change in the Ca\(^{2+}\) sensitivity of tension. We discuss the implications of these results, focusing on the role of strong-binding crossbridges in the regulation of Ca\(^{2+}\) sensitivity of tension.

First, an increase in SL results in a decrease in the lateral separation between the thick and thin filaments in living cardiac muscle.35 Similarly, the interfilament lattice spacing is decreased by increasing SL in skinned (glycerinated) muscle.36 Although the latter study was conducted using skeletal muscle, it is reasonable to assume that the result can be extended to skinned cardiac muscle. In the present study, we observed that increasing SL from 1.9 to 2.3 \(\mu\)m produced about an 11\% reduction in the width of muscle. Thus, it is suggested that there also occurred a corresponding reduction (ie, ≈11\%) in the lattice spacing on extension of SL from 1.9 to 2.3 \(\mu\)m.

On the other hand, 5\% wt/vol dextran decreased the muscle width by ≈13\%. An X-ray diffraction study showed that a reduction in the width of skinned skeletal muscle produced by dextran reflects a proportional change in the lattice spacing.37 Although an X-ray study has not been conducted with cardiac muscle, the conclusion made, on the basis of skeletal muscle, could be applicable to cardiac muscle.38 It can thus be said that in the present study, 5\% wt/vol dextran decreased the lattice spacing by ≈13\%. Given the fact that both an increase in SL and osmotic compression produced a similar reduction in the muscle width and that both increased Ca\(^{2+}\) sensitivity of tension to a similar degree (Figures 1, 2, and 4), we consider that the decreased lattice spacing is the primary mechanism for length-dependent tension generation in skinned cardiac muscle.

There was, however, a slight mismatch between the effect of increasing SL and that of osmotic compression on Ca\(^{2+}\) sensitivity of tension and maximal Ca\(^{2+}\)-activated tension. Increasing SL to 2.3 \(\mu\)m resulted in about an 11\% decrease in the muscle width, whereas osmotic compression decreased the width by ≈13\%, yet the shift of pCa\(_{50}\) and the augmentation of maximal Ca\(^{2+}\)-activated tension were somewhat more pronounced by the lengthening. The exact reason(s) for this mismatch is unknown. However, it may be attributable to...
some direct effect of dextran on the crossbridge cycle and/or to the shape of the muscle being altered differently by mechanical stretch compared with osmotic compression. 

Although Ca$^{2+}$ is a physiological activator of myocardium, it has been known that Ca$^{2+}$ alone does not fully activate the thin filament and that strong-binding crossbridges, such as the rigor complex, can further activate the thin filament. We have reported that the formation of AM.ADP regulates the number of force-generating crossbridges, synergistically with Ca$^{2+}$-binding to TnC. 

In the present study, we found that MgADP, in addition to its apparent Ca$^{2+}$ sensitizing effect, diminished the SL-dependent shift of the pCa-tension relationship in a concentration-dependent manner (Figure 1). Further, MgADP (5 mmol/L) attenuated the increase in Ca$^{2+}$ sensitivity of tension produced by osmotic compression, to a similar degree observed when SL was increased (Figure 4). As discussed above, SL-dependent tension generation can be largely explained due to a decrease in the lattice spacing, which results in an increase in the number of force-generating crossbridges. Therefore, it is realized that when force-generating crossbridges predominate inside the muscle through the cooperative effect of AM.ADP, the effect of lattice shrinkage becomes relatively small, leading to depressed SL-dependent tension generation. This interpretation is consistent with the fact that the potentiating effect of MgADP was significantly less pronounced at a longer SL (Figure 3).

Fitzsimons and Moss reported that an application of N-ethylmaleimide–modified myosin subfragment 1 to single skinned rat ventricular myocytes diminishes the SL-dependent shift of the pCa-tension relationship. It is thus safe to conclude that when the number of force-generating crossbridges is increased through the cooperative effect of strong-binding crossbridges, the effect of increasing SL (ie, lattice shrinkage) to produce force-generating crossbridges is offset.

In skinned muscle preparations, strong-binding crossbridges promote Ca$^{2+}$ binding to TnC. If TnC acts as a “length sensor” in the cardiac contractile system, then it follows that the SL dependence of tension generation would be modulated by a change in the affinity of TnC for Ca$^{2+}$, and the depressed shift of the pCa-tension relationship seen in the presence of MgADP may have been caused by the increased affinity of TnC for Ca$^{2+}$. However, pimobendan was found to have no effect on the SL-dependent shift of the pCa-tension relationship (Figure 2). Thus, it is unlikely that the increased affinity of TnC for Ca$^{2+}$ is the major cause of the attenuation of SL-dependent tension generation by MgADP.

McDonald et al hypothesized that the activation state of muscle with a higher cooperativity varies more dramatically as a result of length-induced variations in the number of force-generating crossbridges. However, in the presence of 0.1 or 1 mmol/L MgADP, the SL dependence was significantly diminished (Figures 1A and 1B), whereas $n_H$ was not significantly changed (Table). Thus, it is unlikely that the attenuation of SL-dependent tension generation by MgADP underlies the decreased cooperative activation of the thin filament.

It should be stressed that MgADP as low as 0.1 mmol/L (or $\approx$0.2 mmol/L when contaminating MgADP is taken into account) augmented maximal and submaximal tension and diminished the SL dependence of tension generation (Figures 1A and 3). Because it has been known that cardiac contractile proteins are more sensitive to MgADP than skeletal muscle proteins, it is possible that MgADP at $\approx$0.1 mmol/L significantly influences cardiac contractile performance, as in the in vitro motility assay system. Recently, Tian et al demonstrated that MgADP significantly increased the left ventricular end-diastolic pressure in intact rat heart. It has been pointed out that in the intracellular milieu of ischemic or hypoxic cardiac muscle, the concentration of ADP increases whereas that of ATP decreases. Reportedly, an increase in the ratio of the concentration of ADP to that of ATP in the vicinity of crossbridges may elicit ischemic contracture. The present results suggest that during ischemia or hypoxia, the accumulation of ADP may impair the Frank-Starling mechanism.

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References


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