Activation of Myocardial Contraction by the N-Terminal Domains of Myosin Binding Protein-C

Todd J. Herron, Elena Rostkova, Gudrun Kunst, Rajiv Chaturvedi, Mathias Gautel, Jonathan C. Kentish

Abstract—Myosin binding protein-C (MyBP-C) is a poorly understood component of the thick filament in striated muscle sarcomeres. Its C terminus binds tightly to myosin, whereas the N terminus contains binding sites for myosin S2 and possibly for the thin filament. To study the role of the N-terminal domains of cardiac MyBP-C (cMyBP-C), we added human N-terminal peptide fragments to human and rodent skinned ventricular myocytes. At concentrations >10 μmol/L, the N-terminal C0C2 peptide activated force production in the absence of calcium (pCa 9). Force at the optimal concentration (80 μmol/L) of C0C2 was ≈60% of that in maximal Ca2+ (pCa 4.5), but the rate constant of tension redevelopment (ktr) matched or exceeded (by up to 80%) that produced by Ca2+ alone. Experiments using different N-terminal peptides suggested that this activating effect of C0C2 resulted from binding by the pro/ala-rich C0-C1 linker region, rather than the terminal C0 domain. At a lower concentration (1 μmol/L), exogenous C0C2 strongly sensitized cardiac myofibrils to Ca2+ at a sarcomere length (SL) of 1.9 μm but had no significant effect at SL 2.3 μm. This differential effect caused the normal SL dependence of myofibrillar Ca2+ sensitivity to be reduced by 80% (mouse myocytes) or abolished (human myocytes) in 1 μmol/L C0C2. These results suggest that cMyBP-C provides a regulatory pathway by which the thick filament can influence the activation of the thin filament, separately from its regulation by Ca2+. Furthermore, the N-terminal region of cMyBP-C can influence the SL-tension (Frank–Starling) relationship in cardiac muscle. (Circ Res. 2006;98:1290-1298.)

Key Words: myosin binding protein C  ■ cardiac myocytes  ■ Ca2+ sensitization  ■ sarcomere length  ■ Frank–Starling mechanism

Myosin binding protein C (MyBP-C) is a myofilament protein of the intracellular immunoglobulin/fibronectin superfamily that is located in the “C-zone” of the sarcomeric A-band in 7 to 9 transverse stripes. Mutations in cardiac MyBP-C (cMyBP-C) constitute a major cause of familial hypertrophic cardiomyopathy,1 yet the functional role of cMyBP-C remains uncertain.2 There is some evidence that cMyBP-C may modulate the dynamics of myocardial contraction, because in skinned myocytes from cMyBP-C knock-out mice, the speed of unloaded and loaded shortening is increased,3 and in working hearts, systole is terminated prematurely.4 In addition, during β-adrenergic stimulation of the heart, cMyBP-C is phosphorylated at a cardiac-specific “motif” near the N terminus. The region around this “motif” binds to myosin S2, near the hinge region of myosin, but when cMyBP-C is phosphorylated, this binding is inhibited.5 This may allow myosin S1 heads to move away from the thick filament, thereby promoting the interaction of S1 with actin.6 On the other hand, in transgenic mice lacking a phosphorylatable form of troponin I, phosphorylation of cMyBP-C alone did not alter the rate of crossbridge cycling in intact muscles or the rate of relaxation in skinned muscles.7

The precise mechanism by which MyBP-C could influence actin–myosin interaction remains to be established. Recent structural evidence from skeletal muscle has suggested that the N-terminal domains of MyBP-C may bind to the thin filament,8 and it has been reported that the N-terminal cardiac C0 domain can bind to actin in solution.9 In the intact myofibril, any such interaction between MyBP-C and thin filament proteins might be expected to vary with the length of the sarcomeres: an increase in sarcomere length (SL) reduces the spacing between thick and thin filaments,10 and this might be predicted to alter the likelihood of MyBP-C binding to the thin filament. Such a SL-dependent action of MyBP-C is of considerable interest because it is well established that an increase in SL enhances the Ca2+ sensitivity of the myofibrils, an effect that is largely responsible for the length–tension (Frank–Starling) relationship in cardiac muscle.10,11 Although the molecular basis for the SL dependence of Ca2+ sensitivity remains controversial, one possibility is that the smaller
spacing between the thick and thin filaments increases the probability of myosin crossbridges attaching to actin at a given concentration of activator Ca$^{2+}$. From the above, it could be predicted that any SL-dependent actions of MyBP-C may modulate, or contribute to, the effect of SL on myofibrillar Ca$^{2+}$ sensitivity.

The aim of the present study was, therefore, to investigate the contribution of the N-terminal domains of cMyBP-C to the SL-dependent regulation of contraction in cardiac muscle. Recombinant N-terminal fragments such as C0C2 (Figure 1A) were applied to human and rodent skinned cardiac myocytes, allowing the exogenous protein to access the myofilaments directly. The effects on myofibrillar force development, crossbridge kinetics, and the SL dependence of Ca$^{2+}$ sensitivity were then determined. We present functional evidence for a new myosin-binding site in the N-terminal of cMyBP-C and show that the N-terminal domains may have a major impact on the magnitude of the Frank–Starling mechanism.

Some of these results have been presented previously in preliminary form.14

Materials and Methods

Expression of Peptides
Truncated N-terminal MyBP-C peptides were prepared by previously described methods.15,16 The cDNA was prepared based on the human MYBC3 sequence. Briefly, soluble MyBP-C fragments were expressed using a pET expression system at 20°C. Following purification the His tag was cleaved from all peptides using TEV protease. Proteins were equilibrated to relaxing (pCa 9.0) or activating solution (pCa 4.5) by dialysis before application to skinned ventricular myocytes.

Myocyte Experiments
Human ventricular biopsies were obtained from transplant donor hearts or patients undergoing corrective cardiac surgery, with full ethical permission, and were frozen in liquid nitrogen. Studies on animals were authorized under the Animals (Scientific Procedures) Act, 1986. The conditions for the care and husbandry of animals were set out in the relevant Codes of Practice issued under the Animals (Scientific Procedures) Act, 1986. Rats (B & K, Hull, UK) and mice (Harlan, Bicester, UK) were killed under pentobarbitone anesthesia and their ventricles were rapidly removed. Myocyte-sized fragments from the human or rodent tissue were then obtained by homogenization in ice-cold relaxing solution.12 Relaxing solution contained (in mmol/L): BES 100, K propionate 55, phosphocreatine 10, MgATP$^2-$ 5, Mg$^{2+}$ 1, dithiothreitol 1, EGTA 10 (replaced by CaEGTA for activating solutions), AEBSF 0.5, leupeptin 0.001, E64 0.001; pH 7.1, ionic strength, 0.20 mol/L. The myocytes were skinned with 1% Triton X-100 in relaxing solution (30 minutes). Preparations consisting of 1 myocyte or a small bundle (~150-μm long and ~35-μm wide) were mounted to a force transducer and high-speed length controller using low-compliance attachments.12,18 Isometric tension and crossbridge cycling kinetics were measured at ~18°C by the method of Brenner and Eisenberg: the myocyte preparation was shortened by 20% in 1 ms and then restretched after 30 ms. This procedure detaches myosin crossbridges from actin, and the rate of tension recovery ($k_t$) after the restretch reflects the rate of crossbridge reattachment and transition to the force-generating state(s).19 The $k_t$ was determined from a single-exponential fit to the force recovery trace. SL in the relaxed myocytes was computed online by Fast-Fourier analysis of the video image of the myocyte (IonOptix, Milton, Mass).

Transfection of MyBP-C Fragments
Cardiac MyBP-C fragments containing domains C0, C0-C1, C1-C2, C0-C2 were phased according to Freiburg and Gautel and cloned into an HA-tagged mammalian expression vector as described previously.21 The constructs were transfected into neonatal rat ventricular myocytes (which can be transfected easily, in contrast to adult myocytes) using standard procedures, and cells were main-
tained as described. The transfected cells were fixed with 4% paraformaldehyde after 2 to 3 days in culture and stained with anti-HA tag antibody (clone 3F10, Roche), anti–myosin heavy chain (MHC) (clone A1025; a gift from S. Hughes, King’s College, London, UK), and Alexa-633 phalloidin (Molecular Probes) for visualization of F-actin. The specimens were analyzed by confocal microscopy using a Zeiss LSM-510 Meta microscope under ×63 magnification and 2 to 3 times zoom.

Results

Activating Effect of C0C2

Following the procedures in our earlier work, in which addition of cardiac C1C2 was found to cause a sensitization of skinned skeletal fibers to calcium, we initially used C0C2 at concentrations between 30 and 80 μmol/L. Whereas in the skeletal fibers we had found no force development at these concentrations of C0C2 if Ca²⁺ was absent (pCa 9), under the same conditions the cardiac myocytes showed a substantial force development (Figure 1B). After C0C2 was applied, force typically took 2 to 4 minutes to reach a new level, which presumably reflects the time taken for the peptide (molecular mass, 49 kDa) to diffuse throughout the preparation. The effect was readily reversible (Figure 1B). This activating effect showed a dose-dependent, sigmoidal relationship in both mouse and human ventricular myocytes (Figure 1C), with EC₅₀ values of 39±4 μmol/L (mean±SD) (n=6) and 37±3 μmol/L (n=6), respectively.

To discover whether the force induced by C0C2 was attributable to normal, cycling crossbridges or to rigor crossbridges, we measured the rate of crossbridge cycling by recording the rate of tension redevelopment (kₜ) following a rapid shortening–restretch maneuver. Figure 2 shows typical results from mouse skinned myocytes in the presence of various concentrations of C0C2 or a maximally activating concentration of Ca²⁺ (pCa 4.5). Maximal Ca²⁺ activated kₜ values reported here are comparable to those previously reported for rodent ventricular myocardium at a similar temperature. Force redevelopment was rapid in the presence of C0C2; indeed, the kₜ value in a maximally activating concentration (80 μmol/L) of C0C2 was similar to that in maximal Ca²⁺ (Figure 2B). The maximum force was, however, significantly smaller with C0C2 than with Ca²⁺. These data indicate that C0C2 initiated the cycling of active, rather than rigor, crossbridges.

The differential effects on force and kₜ suggests that C0C2 was not switching on the thin filament by exactly the same mechanism as Ca²⁺. This was confirmed with myocytes in which troponin C had been extracted by replacing the endogenous troponin complex with recombinant TnT–TnI. After this procedure, the normal activation by Ca²⁺ was lost, but the Ca²⁺-independent activation by C0C2 remained (Figure 2C).

With human myocytes (Figure 3), the kₜ values were lower (presumably largely because of the presence of the slow β-MHC rather than the fast α-MHC in mouse), but the results were similar, except that the differential effects on force and crossbridge-cycling rate were more striking. Although, once again, steady force was less with C0C2 than with maximal Ca²⁺ activation, kₜ for human myocytes in the presence of C0C2 was faster by ~80% than in maximal Ca²⁺ (Figure 3A and 3B). To examine whether this supramaximal effect of C0C2 on crossbridge cycling persisted at different levels of myofibrillar activation, we varied force by adding different concentrations of C0C2 or Ca²⁺ (Figure 3C). The kₜ increased with [Ca²⁺], as seen previously. The kₜ tended to rise with increasing concentrations of C0C2, but even at a [C0C2] giving only ~30% activation of force, the value of kₜ exceeded that at the maximal [Ca²⁺]. This is further evidence that C0C2 and Ca²⁺ activated force production by different mechanisms. The greater effect of C0C2 on kₜ in human
myocytes compared with mouse myocytes (Figure 2B) may be attributable to the fact that the COC2 we used was of the human sequence, which differs by 17% from the mouse COC2 sequence.

**Activation by Different N-Terminal MyBP-C Peptides**

To investigate which regions of COC2 were required for its activating effect, we expressed different N-terminal truncated peptides of cMyBP-C and applied them to human and rodent skinned myocytes (Figure 4). The COC1 fragment (30 μmol/L) acted like COC2 to promote force and crossbridge cycling in the absence of Ca\(^{2+}\), although unlike COC2, maximal force was the same as with Ca\(^{2+}\) activation. In contrast, the C1C2 fragment activated neither force nor crossbridge cycling, as noted previously.\(^{25}\) Similarly, CO was without activating effect on force or \(k_t\). A summary of the activating effects (Figure 4D) illustrates that the critical sequence for this Ca\(^{2+}\)-independent activation appeared to be the pro/ala-rich linker between the CO and C1 domains.

**Localization of the MyBP-C Peptides**

To help identify the proteins that were binding the MyBP-C fragments, we expressed COC1 and COC2 in transfected...
neonatal rat cardiac myocytes (Figure 5). Antibodies specific for these fragments exhibited a striated staining pattern, with C0C1 and C0C2 localized predominantly to the A-band (although there was some fainter, diffuse cytosolic staining, suggesting an unbound fraction). At higher magnification (inset), there was clear indication that these fragments were binding most strongly to the A-band region that contains myosin heads, rather than to the H-zone, which consists of the myosin backbone only. There was no evidence for binding of C0C1 or C0C2 to proteins of the I-band.

**C0C2 Reduces the SL Dependence of Ca\(^{2+}\) Sensitivity**

Previous reports have shown that the Ca\(^{2+}\) sensitivity of cardiac myofibrils is increased by low concentrations (a few micromolar) of fragments C09 or C1C2.\(^{25}\) To see whether the same was true for C0C2, and to study the SL dependence of any effect, we measured myofibrillar Ca\(^{2+}\) sensitivity in the presence of 1 \(\mu\)mol/L C0C2 (a concentration which does not activate force directly at pCa 9; Figure 1) at SLs of 1.9 and 2.3 \(\mu\)m, which span the normal range of diastolic SL in the heart.\(^{11}\) Figure 6 illustrates that raising the SL from 1.9 \(\mu\)m to 2.3 \(\mu\)m increased the Ca\(^{2+}\) sensitivity (as measured by the pCa for 50% force, pCa\(_{50}\)) in human skinned myocytes by 0.13 pCa\(_{50}\) units, consistent with previous studies.\(^{26}\) Mouse myocytes showed a greater SL dependence of Ca\(^{2+}\) sensitivity (results for both species are summarized in Figure 8). As illustrated in Figure 7, the addition of the C0C2 peptide (1 \(\mu\)mol/L) at a submaximal Ca\(^{2+}\) concentration (here pCa 5.6) produced a large increase in force at SL=1.9 \(\mu\)m SL (Figure 7A), but at SL=2.3 \(\mu\)m there was no effect (Figure 7B). This SL-dependent action was seen over the entire range of Ca\(^{2+}\) concentrations. Thus there was a pronounced Ca\(^{2+}\) sensitization by C0C2 at 1.9 \(\mu\)m SL (Figure 7C), increasing pCa\(_{50}\) by 0.29±0.08 pCa units (n=7), whereas there was no significant effect on Ca\(^{2+}\) sensitivity at 2.3 \(\mu\)m SL (Figure 7D; increase in pCa\(_{50}\)=0.07±0.03). There was no significant effect of C0C2 on maximum Ca\(^{2+}\) activated force at either SL (results not shown).

The mean results for human and mouse myocytes (Figure 8A) show that in both species, the large Ca\(^{2+}\) sensitizing action of 1 \(\mu\)mol/L C0C2 at SL=1.9 \(\mu\)m was abolished at 2.3 \(\mu\)m. It followed from this that the normal SL dependence of myofibrillar Ca\(^{2+}\) sensitivity (eg, Figure 6) would be different in the absence and presence of C0C2. In Figure 8B, we replot the data to show how the presence of C0C2 altered the SL dependence of myofibrillar Ca\(^{2+}\) sensitivity. The increase in pCa\(_{50}\) (0.13±0.04) seen when the SL was increased from 1.9 \(\mu\)m to 2.3 \(\mu\)m in human myocytes was, in fact, completely abolished by the addition of C0C2; the corresponding stretch-induced increase in pCa\(_{50}\) in mouse myocytes was larger (0.23±0.05) than in human myocytes but was reduced to only \(\approx\)20% of this value if C0C2 was present. Thus the exogenous N-terminal fragment of MyBP-C had profound inhibitory effects on the SL dependence of myofibrillar Ca\(^{2+}\) sensitivity.
Discussion

In the present work, we show that N-terminal fragments of cMyBP-C can affect force production and crossbridge activity in skinned myocyte preparations from rodent and human ventricles in a previously unrecognized way. At concentrations \(10^{-10}\) mol/L, C0C2 (and C0C1) induced a Ca\(^{2+}\)-independent activation of crossbridge cycling and force development. At a lower concentration (1 \(10^{-9}\) mol/L), C0C2 increased myofibrillar Ca\(^{2+}\) sensitivity at a SL of 1.9 \(\mu\)m, but this effect was absent at 2.3 \(\mu\)m; this caused the normal SL dependence of myofibrillar Ca\(^{2+}\) sensitivity to be virtually abolished in the presence of C0C2. This SL-dependent effect suggests that MyBP-C may modulate the SL dependence of myofibrillar Ca\(^{2+}\) sensitivity.

Activation of Crossbridge Cycling in the Absence of Ca\(^{2+}\)

Although it is commonly accepted that Ca\(^{2+}\) is required to switch on crossbridge cycling and force development, we found that the addition of C0C2 or C0C1 at concentrations above 10 \(\mu\)mol/L induced a large activation of force development in the virtual absence of Ca\(^{2+}\) (pCa 9). To our knowledge, this is the first report that a thick filament protein (other than myosin S1 when [MgATP] is low) can switch on crossbridge cycling in the absence of Ca\(^{2+}\). This action was not seen in previous studies using N-terminal fragments of cMyBP-C, but these studies used fragments containing either C09 or C1C2,\(^{25}\) neither of which exhibit this Ca\(^{2+}\)-independent activation (Figure 4). One similarity between activation by C0C2 and Ca\(^{2+}\) was that the relationship between force and [C0C2] was sigmoidal (Figure 1), as it is for Ca\(^{2+}\). Compared with the normal activation by Ca\(^{2+}\), however, the activation by C0C2 achieved a smaller maximum force but a similar or even supramaximal rate of crossbridge cycling (Figure 2 and 3). In addition, the activating effect of C0C2 also occurred in TnC-extracted preparations, in which Ca\(^{2+}\) activation was abolished (Figure 2C). These differential effects suggest that C0C2 was switching on the thin filament by mechanism different from that of Ca\(^{2+}\).

In terms of the apparent rates of crossbridge attachment (\(f_{app}\)) and detachment (\(g_{app}\)) in a 2-state model of the crossbridge cycle,\(^{19}\) the actions of C0C2 on force and \(k_a\) (which is
proportional to \( f_{app} + g_{app} \) may be explained if C0C2 not only increases \( f_{app} \) as \( Ca^{2+} \) does,\(^{19}\) but also increases \( g_{app} \) (thereby tending to increase \( k_c \), but decrease force).

Because only approximately one-third of myosin molecules (ie, those in the C-zone of the sarcomere) have endogenous cMyBP-C bound, it is likely that the exogenous cMyBP-C fragments were activating contraction by binding to vacant cMyBP-C-binding sites and mimicking the actions of the endogenous cMyBP-C, rather than by competing with endogenous cMyBP-C for its usual binding sites. If the activation was being caused by a competition effect, then the endogenous cMyBP-C would have to be exerting a strong inhibition of crossbridge activation. This seems highly unlikely, because in cMyBP-C null mice there is no maintained activation of the myofibrils if \( Ca^{2+} \) is absent;\(^{25}\) in addition, adding the human C1C2 peptide increased myofibrillar \( Ca^{2+} \) sensitivity to the same extent in skinned myocytes from wild-type and cMyBP-C knockout mice, indicating that this could not be a competition effect.\(^{25}\)

The results using various MyBP-C fragments (Figure 4) provide information on the critical region of cMyBP-C needed for the \( Ca^{2+}\)-independent activation of crossbridge cycling. Whereas previous binding studies have suggested that the N-terminal region of cMyBP-C can bind to myosin S2 via the region around the cMyBP-C motif,\(^{15}\) or to actin via C0,\(^{9}\) our data suggest that neither of these domains is required for the activating effect of exogenous C0C2. The C0C1 fragment, which lacks the MyBP-C motif and does not bind myosin S2 with high affinity,\(^{15}\) acted like C0C2 to switch on force and crossbridge cycling (Figure 4), whereas the C1C2 fragment, which does contain the myosin S2 binding site, activated neither force nor crossbridge cycling in the absence of \( Ca^{2+}\), as noted previously.\(^{24}\) Neither did the N-terminal C0 domain itself have an activating effect (Figure 4). Rather, the critical region for activation appeared to be the pro/ala-rich linker between the C0 and C1 domains (Figure 4D). It may be noted that the C0 fragment used by Kulikovskaya et al\(^{10}\) contained, in fact, 54 additional residues of this pro/ala-rich linker region.

The precise mechanism for the activating effect of C0C1 and C0C2 on force production and crossbridge cycling remains to be established. Theoretically, the \( Ca^{2+}\)-independent crossbridge cycling might be induced by the cMyBP-C fragments either binding to the thin filament and switching it on directly or binding to the thick filament to promote the attachment of myosin S1 heads to actin in sufficient numbers to activate the thin filament (as occurs during the development of rigor). The labeling studies (Figure 5) demonstrated binding of the fragments to the thick filament crossbridge region, presumably to myosin. This A-band localization is to be expected for C0C2, because the C1C2 part of this fragment contains the motif region that binds to myosin S2.\(^{15}\) The A-band localization of C0C1 suggests that C0C1 and C0C2 contain a novel region, presumably the pro/ala-rich sequence (Figure 4), that binds to a thick filament protein. Flavigny et al\(^{27}\) also found that exogenous C0C1 was restricted to the A-band and suggested that this was caused by C0 binding to myomesin-binding sites on myosin. However, the C0 fragment alone had no activating effect in our experiments (Figure 4) and localized diffusely in the transfected ventricular cells (results not shown). Interestingly, a pro/ala-rich region homologous to that in cMyBP-C is found in the N-terminal extension of the A1 type of essential light chain (ELC-1),\(^{8}\) as found in cardiac myosin. Recent high-resolution structural studies of thick filaments have shown interactions between the ELC bound to the neck region of one myosin S1 head and the motor domains of other S1 heads; these interactions could keep the myosin heads close to the filament backbone in the relaxed muscle.\(^{28}\) The N-terminal homology of cMyBP-C with ELC-1 raises the possibility that the pro/ala-rich sequence of the C0C1 domain could, by interfering with these interactions between ELC-1 and the S1 heads, free some of the heads and increase their flexibility, thereby promoting the binding of S1 to actin. This action would tend to increase myofibrillar \( Ca^{2+}\) sensitivity at low concentrations of cMyBP-C (Figure 7) and might account for the activation of the thin filament at higher concentrations of cMyBP-C.

The confocal images of the transfected cells suggest that C0C1 and C0C2 bind with high affinity to the thick filament. However, our data do not exclude the possibility that these cMyBP-C domains could bind additionally to the thin filament, although with much lower affinity. A direct interaction between the N-terminal regions of MyBP-C and actin was suggested by structural evidence from fish skeletal muscle\(^{8}\) and from binding studies with cardiac MyBP-C.\(^{9}\) The homologous N-terminal fragments of ELC-1 are reported to bind to actin and have been shown to alter crossbridge kinetics, raise the \( Ca^{2+}\) sensitivity of force in skinned fibers, and induce a supramaximal activation of \( Ca^{2+}\)-activated actomyosin ATPase activity (reviewed by Schaub et al\(^{29}\)). From the homology between the N-terminal regions of the proteins, it was predicted that MyBP-C could exert effects similar to ELC.\(^{8}\) However, binding to actin alone cannot explain the lack of thin filament binding of the cMyBP-C fragments (Figure 5), nor the muscle-type specificity that we observed: we found no activating effect of the N-terminal slow skeletal nC2 peptide on cardiac myofibrils (Figure 4) nor of the cardiac C0C2 peptide on mouse skinned soleus fibers (not shown), yet actin is virtually identical in cardiac and slow skeletal muscle (98% amino acid identity and 100% homology) and so is unlikely to confer this pronounced muscle-type specificity.

We conclude that our data may be explained if the pro/ala-containing C0C1 sequence of cMyBP-C binds to myosin and promotes crossbridge binding to actin. A myosin-based mechanism can more readily account for the observed supramaximal stimulation of the crossbridge cycling rate (Figures 2 and 3). There may be additional binding of cMyBP-C to actin at higher concentrations of cMyBP-C, although this is likely to be weak and labile, as suggested previously;\(^{8}\) nevertheless, this could aid the activation of crossbridge cycling in the absence of \( Ca^{2+}\). Further work is needed to clarify the binding partner(s) for these MyBP-C fragments.

**SL-Dependent Sensitizing Action of C0C2**

At a lower concentration (1 \( \mu \text{mol/L} \)), C0C2 increased the \( Ca^{2+}\) sensitivity of the myofibrils markedly at SL 1.9 \( \mu \text{m} \), but,
surprisingly, this sensitization was absent at a SL of 2.3 μm (Figure 7). A corollary of this strong interaction between the effects of C0C2 and SL on myofibrillar Ca2+ sensitivity was that this low concentration of C0C2 reduced or abolished the SL dependence of Ca2+ sensitivity (Figure 8). One possible mechanism for the SL dependence of Ca2+ sensitivity is that as the sarcomere is lengthened, the smaller spacing between thick and thin filaments enhances the probability of actin–myosin interaction.10,12 Consistent with this, interventions such as lowering theionic strength10 or adding NEM-S1,12 which increase the number of weakly or strongly bound crossbridges interacting with actin, have been reported to reduce or abolish the SL dependence of Ca2+ sensitivity. It is postulated that, by promoting actin–myosin interaction at shorter SLs, these interventions replicate the effect of increasing SL (reviewed by Fuchs and Martyn13). It may be that COC2 studied here has a similar action, ie, COC2 increases myofibrillar Ca2+ sensitivity at SL=1.9 μm by binding to myosin and increasing the freedom and/or flexibility of myosin heads, allowing them to interact more readily with actin, whereas at SL=2.3 μm the spacing between S1 heads and actin is already small enough for optimal interaction, thus resulting in little further effect of adding the cMyBP-C fragment. Thus these data provide evidence that, even at low concentrations, the N-terminal region of MyBP-C can modulate the interaction of myosin with actin, probably by altering the availability of crossbridges able to interact with the thin filament. We predict that the N-terminal sequence of endogenous cMyBP-C within the myofibril may also exert a similar effect. The SL dependence of this effect suggests that endogenous cMyBP-C could potentially modulate the length–tension relationship in cardiac muscle. Consistent with this, a recent study has found a reduced SL dependence of Ca2+ sensitivity in the hearts of cMyBP-C knockout mice.31

In summary, our results provide evidence for a novel interaction between the pro/ala-rich sequence of the N-terminal cMyBP-C region and an A-band protein, almost certainly a myosin component. This N-terminal region can (at higher concentrations) activate crossbridge cycling independently of Ca2+ and (at lower concentrations) promote the Ca2+ activation of crossbridge cycling in a SL-dependent manner. In the intact myocyte, the average concentration of cMyBP-C is ≈8 μmol/L.25 However, because cMyBP-C is localized to the sarcomeric C-zone, its local concentration will be more than double this, and thus within the range for possible Ca2+–independent activation (Figure 1). On the other hand, steric constraints on the motion of the endogenous, bound cMyBP-C would reduce the effective concentration seen by other myofibrillar proteins, perhaps to a few micromolar. Although the precise effective concentration of cMyBP-C in the sarcomeric C-zone remains uncertain, our results suggest that the N-terminal region of endogenous MyBP-C within the C-zone may function to modulate the Ca2+ activation of crossbridge cycling in cardiac myofibrils.

Acknowledgments
This work was supported by grants from the Medical Research Council (to M.G. and J.C.K.). We are grateful to E. Ehler for providing neonatal cardiomyocytes.

References
3. Korte FS, McDonald KS, Harris SP, Moss RL. Loaded shortening, power output, and rate of force development are increased with knockout of cardiac myosin binding protein-C. Circ Res. 2003;93:752–758.

Downloaded from http://circres.ahajournals.org/ by guest on April 2, 2017


Activation of Myocardial Contraction by the N-Terminal Domains of Myosin Binding Protein-C

Todd J. Herron, Elena Rostkova, Gudrun Kunst, Rajiv Chaturvedi, Mathias Gautel and Jonathan C. Kentish

Circ Res. 2006;98:1290-1298; originally published online April 13, 2006;
doi: 10.1161/01.RES.0000222059.54917.ef

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/98/10/1290

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/