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Power Output Is Increased After Phosphorylation of Myofibrillar Proteins in Rat Skinned Cardiac Myocytes

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Abstract—β-Adrenergic stimulation increases stroke volume in mammalian hearts as a result of protein kinase A (PKA)-induced phosphorylation of several myocyte proteins. This study investigated whether PKA-induced phosphorylation of myofibrillar proteins directly affects myocyte contractility. To test this possibility, we compared isometric force, loaded shortening velocity, and power output in skinned rat cardiac myocytes before and after treatment with the catalytic subunit of PKA. Consistent with previous studies, PKA increased phosphorylation levels of myosin binding protein C and troponin I, and reduced Ca^{2+} sensitivity of force. PKA also significantly increased both maximal force (25.4±8.3 versus 31.6±11.3 μN [$P<0.001$, n=12]) and peak absolute power output (2.48±1.33 versus 3.38±1.52 μW/mg [$P<0.05$, n=5]) during maximal Ca^{2+} activations. Furthermore, PKA elevated power output at nearly all loads even after normalizing for the increase in force. After PKA treatment, peak normalized power output increased ≈20% during maximal Ca^{2+} activations (n=5) and ≈33% during half-maximal Ca^{2+} activations (n=9). These results indicate that PKA-induced phosphorylation of myofibrillar proteins increases the power output–generating capacity of skinned cardiac myocytes, in part, by speeding the step(s) in the crossbridge cycle that limit loaded shortening rates, and these changes likely contribute to greater contractility in hearts after β-adrenergic stimulation. **(*Circ Res*. 2001;89:1184-1190.)**

Key Words: cardiac myocytes ■ β-adrenergic stimulation ■ cardiac contractility ■ sarcomere proteins ■ protein kinase A

Myocardial performance is enhanced when β-adrenergic receptors are stimulated by catecholamines. After β-adrenergic receptor stimulation, myocardial performance is associated with increased force development and faster rates of both the rise and fall of force.[1] These positive inotropic and lusitropic effects are mediated by 3′-5′ cAMP-dependent protein kinase (protein kinase A [PKA]), which phosphorylates several proteins inside cardiac myocytes including the sarcolemmal Ca^{2+} channel, the ryanodine receptor,[2] phospholamban, troponin I (TnI), and myosin binding protein C (MyBP-C).[3] Four of these phosphoproteins (the sarcolemmal Ca^{2+} channel, ryanodine receptor, phospholamban, and TnI) are involved with the handling of intracellular Ca^{2+}, and the phosphorylation state of these proteins regulates both the amplitude and duration of the Ca^{2+} transient. Therefore, it is generally accepted that Ca^{2+} handling is an important molecular mechanism underlying the inotropic effects of β-adrenergic receptor stimulation.[4,5] However, changes in Ca^{2+} handling may not be the sole mechanism responsible for the inotropic effects of β-adrenergic receptor stimulation, given that the rise and fall of the Ca^{2+} transient and the binding of Ca^{2+} to troponin C all appear to be too rapid to limit the rise and fall of pressure during a heartbeat.[6] Thus, the factors that determine the rise and fall of pressure during a heartbeat also likely reside, at least in part, within the myofibrillar proteins.

Phosphorylation of myofibrillar proteins (ie, MyBP-C and TnI) by PKA may mediate inotropic effects of β-adrenergic stimulation by a mechanism independent of changes in intracellular Ca^{2+} handling, such as by directly modulating the rate of crossbridge cycling. However, variable responses in crossbridge cycling rates have been observed after β-adrenergic stimulation of myocardial preparations. The β-adrenergic receptor agonist isoproterenol was found to increase the frequency for minimum stiffness in papillary muscles during isometric barium contractures; this was interpreted to reflect an increase in isometric crossbridge cycling rates.[7,8] On the other hand, myofibrillar ATPase activity was unaltered during Ca^{2+}-induced isometric contractions after PKA treatment of skinned cardiac trabeculae,[9] suggesting that β-adrenergic stimulation may not alter overall crossbridge cycling kinetics during isometric contractions. Variable results have also been reported from studies that examined the effect of β-adrenergic stimulation on the unloaded shortening velocity (V_o) of myocardial preparations. Isoproterenol did not speed V_o of sarcomere shortening at optimal $[Ca^{2+}]_o$ in

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TABLE 1. Summary of Myocyte Dimensions

		Sarcomere Length	
Length, μm	Width, μm	pCa 9.0, μm	pCa 4.5, μm
153±54	21±4	2.29±0.08	2.24±0.05

Values are mean±SD; n=12.

intact rat cardiac trabeculae preparations.[10] In addition, PKA has been reported to have no effect on V_o in either skinned cardiac trabeculae[11] or single cardiac myocyte preparations[12] during maximal Ca^{2+} activations. Contrary to these results, PKA has been observed to speed V_o in single skinned cardiac myocytes.[13,14] We did not attempt to resolve these discordant results on isometric properties and unloaded shortening velocities, but rather we studied the effect of PKA on cross-bridge cycling rates during loaded contractions, which mimics the way myocytes function during the ejection phase of the cardiac cycle.

Thus, the purpose of this study was to determine whether PKA-induced phosphorylation of myofibrillar proteins alters loaded shortening velocity and power output of single skinned cardiac myocytes. To this end, loaded shortening velocities and power output were measured in single permeabilized rat cardiac myocytes before and after treatment with the catalytic subunit of PKA.

Materials and Methods

Cardiac Myocyte Preparation

Cardiac myocyte preparations were obtained by mechanical disruption of hearts from male 2- to 3-month-old Sprague-Dawley rats as described previously.[15] Rats were obtained from Harlan Tekland (Madison, Wis) and all animal usage was performed according to guidelines established by the Animal Care and Use Committee of the University of Missouri. Dimensions of the myocyte preparations are listed in Table 1. Rats were housed in groups of 2 or 3 and were provided access to food and propranolol (1 mg/mL)-treated water ad libitum. Propranolol is a β-adrenergic antagonist and was administered in an attempt to normalize baseline PKA-induced phosphorylation levels of myofibrillar proteins in each animal. The experimental apparatus for physiological measurements on myocyte preparations was similar to one previously described in detail.[15,16]

Compositions of relaxing and activating solutions were as follows (in mmol/L): EGTA 7, free Mg^{2+} 1, imidazole 20, MgATP 4, and creatine phosphate 14.5 (pH 7.0); various Ca^{2+} concentrations between 10^{-9} (relaxing solution) and $10^{-4.5}$ mol/L (maximal Ca^{2+} activating solution); and sufficient KCl to adjust ionic strength to 180 mmol/L.[15] All chemicals were of the highest purity available and obtained from Sigma.

Force-Velocity and Power-Load Measurements

All mechanical measurements were made at 13±1°C. The protocol for force-velocity and power-load measurements has been previously described in detail.[17] Force-velocity and power-load measurements were made on each myocyte before and after treatment with the catalytic subunit of bovine PKA (3 to 5 μg/mL). First, the myocyte was transferred into maximal Ca^{2+} activating solution, and once steady-state force was attained, a series of force clamps was performed to determine isotonic shortening velocities. Using a servo system, force was maintained constant for a designated period of time (150 to 250 ms) while the length change was continuously monitored. After the force clamp, the myocyte preparation was slackened to reduce force to near zero to allow estimation of the relative load sustained during isotonic shortening; the myocyte was subsequently re-extended to its initial length. An example of a series of force clamps is shown in Figure 1. After the first series of force clamps, the myocyte was treated with the catalytic subunit of PKA for 45 minutes and loaded shortening velocities were again measured. Force-velocity relationships were also obtained before and after PKA treatment in a separate group of myocytes during half-maximal Ca^{2+} activations. A third group of force-velocity relationships were obtained during half-maximal Ca^{2+} activations before and after incubation of myocytes with PKA plus bovine protein kinase inhibitor (PKI, ≈300 μg/mL) to test the specificity of post-PKA responses.

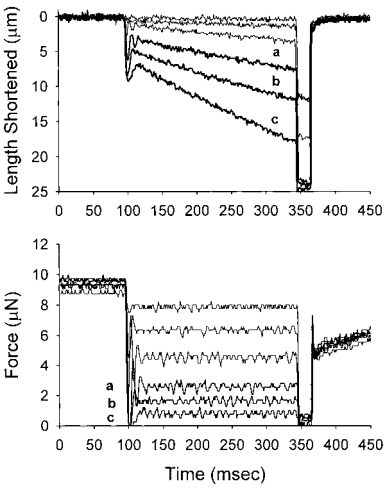

Figure 1. Length traces (top) during a series of force clamps imposed on a single myocyte preparation during maximal Ca^{2+} activation. Velocity of shortening during each load clamp was taken at the onset of force clamp as described in Materials and Methods. Length traces labeled a, b, and c correspond to force clamps a, b, and c.

Data Analysis

Myocyte preparation length traces, force-velocity curves, and power-load curves were analyzed as previously described.[18] Paired *t* tests were used to determine whether there were significant differences in force-velocity parameters before and after PKA treatment.

SDS-PAGE and Autoradiography

To determine myofibrillar substrates of PKA, myofibrillar samples were incubated with the catalytic subunit of PKA in the presence of radiolabeled ATP, separated by SDS-PAGE, and visualized by autoradiography. Briefly, skinned cardiac myocytes (100 μg) were incubated with the catalytic subunit of PKA (3 to 5 μg/mL) and 50 μCi [γ-^{32}P]ATP for 30 minutes. The reaction was stopped by the addition of electrophoresis sample buffer and heating at 95°C for 3 minutes. The samples were then separated by SDS-PAGE, silver stained, dried, and subsequently exposed to x-ray film for ≈1 hour at −70°C. Figure 2 demonstrates that two myofibrillar proteins (MyBP-C and TnI) were phosphorylated by PKA, and the apparent level of baseline phosphorylation of these two proteins was reduced at the time of euthanasia in animals given the oral β-blocker propranolol (lane 2).

Results

The effects of PKA on myocyte force, shortening velocity, and power output are summarized in Table 2 and Figure 3. After PKA treatment, skinned myocytes generated ≈25% more force during maximal Ca^{2+} activation (ie, pCa 4.5). The

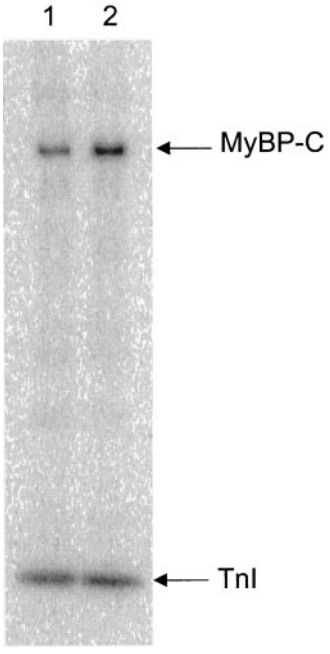

Figure 2. Autoradiogram showing phosphate incorporation into MyBP-C and cardiac TnI after PKA treatment in skinned cardiac myocytes. Lane 1 contains skinned cardiac myocytes (17 μg) obtained from a control rat, whereas lane 2 contains myocytes from a rat pretreated with the oral β-blocker propranolol. Pretreatment with propranolol reduced the apparent level of baseline phosphorylation, as densitometric analysis indicated ≈1.5 and 3 times greater PKA-induced phosphorylation of TnI and MyBP-C, respectively.

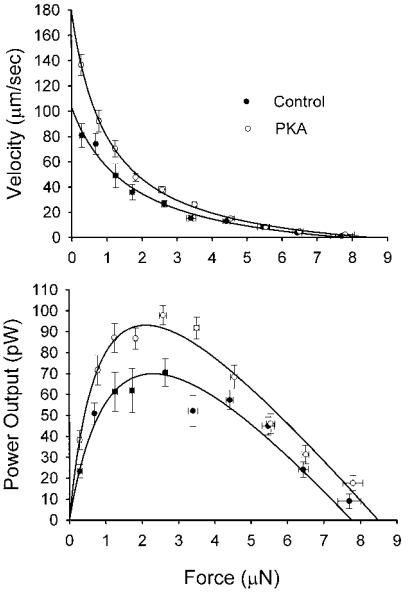

Figure 3. Summary of effects of PKA on force-velocity and absolute power-load curves in cardiac myocyte preparation (n=5) during maximal Ca^{2+} activations. After PKA treatment, there was an increase in both maximal force and power output. The increase in force in this figure underestimates the actual increase in force when measured directly (see values in Tables 2 and 3). Bars indicate SEs for both force (horizontal bars) and power output (vertical bars).

top panel of Figure 3 shows that PKA shifted the absolute force-velocity curves upward, such that the mean velocity of shortening is greater at each given absolute load. For example, before PKA treatment, velocity of shortening was ≈48 μm/s (≈0.31 muscle length (ML)/s when the absolute load was 1 μN, but after PKA treatment, velocity of shortening increased to ≈71 μm/s (≈0.46 ML/s) at the same absolute load. Based on force-velocity curves, maximum velocity of shortening (V_{max}) increased 51% in response to PKA (for control, 0.99±0.27 ML/s; for PKA, 1.49±0.35 ML/s [$P<0.05$]) and mean shortening velocity at loads optimal for power output (V_{opt}) was 37% faster after PKA treatment (for control, 0.27±0.05; for PKA, 0.37±0.06 ML/s [$P<0.05$]) (Table 2). The bottom panel of Figure 3 shows the effect of PKA treatment on absolute power-load curves. PKA treatment increased the power output–generating capacity at each absolute load less than isometric. PKA increased peak absolute power output by 34% during maximal Ca^{2+} activation (for control, 70±22 pW; for PKA, 94±19 pW [$P<0.05$]).

The increased peak absolute power output after PKA treatment may be attributed to greater force-generating capacity, so that a given load became a lesser relative load, which allowed the myocyte to shorten faster in accordance with the force-velocity relationship. Alternatively, PKA-induced phosphorylation of myofibrillar proteins may have directly sped loaded crossbridge cycling rates, such that velocity of shortening was faster at the same relative load. To

TABLE 2. PKA Effects on Maximal Ca^{2+} Activated Force, Velocity, and Peak Power Output

	Before PKA	After PKA
Maximum force, μN	7.0±1.8	8.6±1.7*
Maximum force, kN · m^{-2}	29.7±7.3	37.5±10.1*
V_{max}, ML · s^{-1}	0.99±0.27	1.49±0.35*
V_{opt}, ML · s^{-1}	0.27±0.05	0.37±0.06*
F_{opt}	0.28±0.03	0.25±0.03
Peak absolute power output, pW	70.3±21.7	94.2±19.3*
Peak power output, μW · mg^{-1}	2.48±1.33	3.38±1.52*
Peak normalized power output, P/P_o · ML · s^{-1}	0.077±0.006	0.092±0.014*

Values are mean±SD; n=5. F_{opt} indicates relative force at which power was optimal. *Significant difference from before PKA; $P<0.05$.

TABLE 3. PKA Effects on Half-Maximal Ca^{2+} Activated Force, Velocity, and Peak Power Output

	Before PKA	After PKA
Maximum force, μN	8.2±9.7	10.0±5.62*
Maximum force, kN · m^{-2}	22.2±7.5	27.2±10.0*
V_{max}, ML · s^{-1}	0.50±0.13	0.61±0.11*
V_{opt}, ML · s^{-1}	0.17±0.04	0.22±0.03*
F_{opt}	0.34±0.04	0.36±0.05
Peak absolute power output, pW	41.4±39.7	67.2±47.5*
Peak power output, μW · mg^{-1}	0.72±0.35	1.17±0.45*
Peak normalized power output, P/P_o · ML · s^{-1}	0.057±0.016	0.076±0.014*

Values are mean±SD; n=9. *Significant difference from before PKA; $P<0.05$.

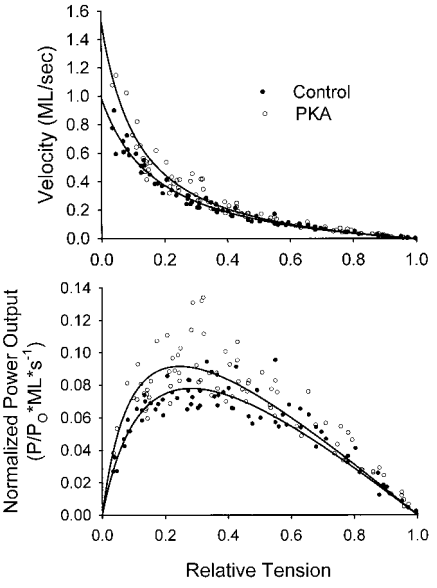

Figure 4. Cumulative force-velocity and power-load curves normalized for isometric force before and after PKA treatment of cardiac myocyte preparations (n=5). These curves were obtained from preparations that were maximally Ca^{2+} activated. Peak normalized power output increased ≈20% after PKA treatment.

address whether the greater absolute power output resulted from greater force-generating capacity or faster loaded shortening, force-velocity and power-load curves were normalized to isometric force before and after PKA treatment (Figure 4). After PKA treatment, the velocity of myocyte shortening was greater at relative loads $<\approx$70% isometric force, suggesting faster crossbridge cycling after phosphorylation of MyBP-C and TnI. The bottom panel of Figure 4 shows that normalized power output was greater at nearly all relative loads, and peak normalized power output was ≈20% greater (0.077 ± 0.006 versus 0.092 ± 0.014 $P/P_o \cdot ML \cdot s^{-1}$) after PKA treatment. These results suggest that phosphorylation of myofibrillar proteins by PKA increases absolute power output of single myocytes by both increasing force-generating capacity, such that a given absolute load is a lesser fraction of isometric force, and by increasing loaded shortening rates at most relative loads, which likely results from faster rates of crossbridge cycling under load.

Because Ca^{2+} activation of myofilaments does not likely reach maximum in vivo, we also examined the effects of PKA on force, velocity, and power during half-maximal Ca^{2+} activations. These effects are summarized in Table 3. After PKA treatment, Ca^{2+} sensitivity of force was reduced such that the pCa solution that yielded half-maximal activation shifted from 5.64 ± 0.08 before PKA treatment to 5.53 ± 0.08 after PKA treatment ($P<0.001$, paired t test). PKA treatment also shifted normalized force-velocity and power-load curves upward during half-maximal Ca^{2+} activations (Figure 5). Interestingly, during half-maximal Ca^{2+} activation, the PKA-induced increase in peak normalized power output was greater than that observed during maximal Ca^{2+} activations (33% versus 19%). Also, during half-maximal Ca^{2+} activations, normalized power output was greater at all relative loads and, in fact, the difference in power output before and after PKA treatment increased as relative load increased until finally converging at isometric force, where there was no shortening. Overall, these results imply that loaded crossbridge cycling is faster after phosphorylation of MyBP-C and TnI during half-maximal Ca^{2+} activation, and this effect is relatively greater than that observed during maximal Ca^{2+} activation.

A final series of control experiments was performed by incubating myocytes in PKA plus PKI. These four myocytes had preparation lengths of 150 ± 25 μm and widths of 19 ± 3 μm. After incubation of the myocytes in PKA+PKI, there was no increase in force during maximal Ca^{2+} activation (force before PKA+PKI=8.8 ± 1.6 μN; force after PKA+PKI=8.1 ± 2.1 μN [$P=0.10$]). Additionally, force-velocity and power-load curves were similar before and after treatment with PKA+PKI during half-maximal Ca^{2+} activations. Peak normalized power output was 0.073 ± 0.019 before and 0.067 ± 0.014 $P/P_o \cdot ML \cdot s^{-1}$ after PKA+PKI, respectively ($P=0.21$). These results are consistent with the conclusion that changes in skinned myocyte contractility after PKA treatment arise from PKA-induced phosphorylation of MyBP-C and/or TnI.

Discussion

We examined the effects of PKA-induced phosphorylation of cardiac myofibrillar proteins on isometric and isotonic contractions of single skinned cardiac myocytes. Maximal Ca^{2+}-activated force, peak absolute power, and peak normalized power were all significantly greater after PKA treatment. Because peak power was greater after PKA treatment even when normalized for changes in force, we conclude that PKA increased absolute peak power, in part, by speeding loaded crossbridge cycling rates. These results

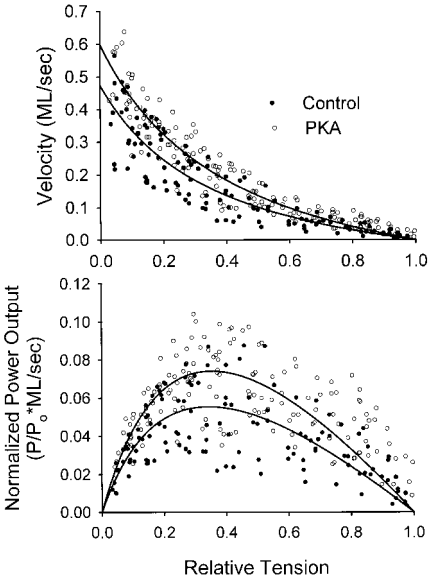

Figure 5. Cumulative force-velocity and normalized power-load curves obtained during half-maximal Ca^{2+} activations of cardiac myocyte preparations before and after PKA treatment (n=9). Peak normalized power output increased ≈30% after PKA treatment.

provide evidence that the enhancement of myocardial performance by β-adrenergic stimulation may be mediated partly through effects of phosphorylation of myofibrillar proteins to accelerate the transition steps in the crossbridge cycle that limit power output.

Effects of β-Adrenergic Stimulation on Myofilament Function

β-Adrenergic stimulation of intact hearts or PKA treatment of skinned myocardium phosphorylates two myofibrillar proteins, MyBP-C and TnI, in mammalian hearts.[3] A variety of experimental approaches have examined whether and how PKA-induced phosphorylation of TnI and MyBP-C alters cardiac myofilament function. These studies have for the most part addressed the effects of phosphorylation on isometric contractile properties and unloaded shortening velocities. Regarding isometric parameters, both β-adrenergic receptor stimulation of intact myocardium[4] and PKA treatment of skinned myocardium[9,11–13,19,20] have been reported to significantly reduce the Ca^{2+} sensitivity of myofilament function. This effect appears to be due to phosphorylation of two serine residues in the N-terminal region of cardiac TnI,[21] which lowers the affinity of TnC for Ca^{2+}.[19,22] It has been suggested that such a reduction in Ca^{2+} sensitivity of myofilament function may work to speed isovolumic relaxation of the heart during β-adrenergic stimulation, which would allow adequate filling of the ventricles during diastole.[20] Aside from reducing Ca^{2+} binding affinity of TnC, PKA-induced phosphorylation of myofibrillar proteins could also influence myocardial function by altering crossbridge cycling rates. Consistent with this idea, experiments on intact rabbit and rat papillary muscles demonstrated that the frequency of minimum stiffness was increased after β-adrenergic stimulation during Ba^{2+} contractures.[7,8] These results indicated that the β-adrenergic–induced acceleration of isometric crossbridge cycling rates occurs independently of any changes in cytosolic Ca^{2+} handling and, thus, were the result of phosphorylation of TnI and/or MyBP-C. Consistent with these results, isometric relaxation rates after rapid chelation of Ca^{2+} were accelerated after PKA treatment of skinned porcine[23] and murine[24] myocardial preparations. These results were also interpreted as an acceleration of isometric crossbridge cycling rates in response to phosphorylation of myofibrillar proteins. In contrast, however, de Tombe and Steinen[9] found no change in isometric myofibrillar ATPase activity during maximal Ca^{2+}-activated isometric contractions after PKA treatment of skinned rat trabeculae, which implies no change in Ca^{2+}-activated isometric crossbridge cycling rates. Additional studies have investigated the effects of β-adrenergic stimulation on unloaded muscle shortening. de Tombe and ter Keurs[10] showed that unloaded sarcomere shortening increased as a sigmoid function of isoproterenol concentration in intact rat trabeculae preparations. However, isoproterenol did not increase unloaded shortening at optimal $[Ca^{2+}]_o$, which implies that β-adrenergic stimulation only speeds crossbridge cycling rates secondary to its effects on Ca^{2+} handling in these preparations. Other studies have similarly reported no change in unloaded shortening rates in either rat skinned trabeculae preparations[11] or rat skinned cardiac myocyte preparations[12] after PKA treatment. Because mean strain on crossbridges is likely to be lower during unloaded contractions compared with isometric contractions, these results have been reconciled with previous results from isometric Ba^{2+} contractures experiments by suggesting that β-adrenergic stimulation alters crossbridge cycling rates in a strain-dependent manner. However, Strang et al[13] observed that both isoproterenol treatment of intact myocytes before skinning and PKA treatment of skinned myocytes caused a ≈40% increase in unloaded shortening rates. A similar increase in unloaded shortening rates was reported after PKA treatment of single skinned mouse cardiac myocytes.[14]

Overall, it is clear that PKA has elicited highly variable experimental responses on cardiac myofilament function, and these differences are difficult to reconcile. Although the exact reasons for these differences are unknown, they certainly may arise from differences in types of animals or in myocardial preparations (eg, multicellular or single cell), variations in contractile protein isoform expression, and/or variable levels of baseline phosphorylation of myofibrillar proteins. Regarding the latter possibility, we attempted to minimize MyBP-C and TnI phosphorylation levels before PKA treatment by treating animals with the β-blocker propranolol well in advance of euthanasia. Based on autoradiography, this appeared to reduce baseline phosphorylation of MyBP-C and TnI, as indicated by greater PKA-induced phosphorylation of MyBP-C and TnI from propranolol-treated animals (Figure 2). This may explain the rather robust changes in myocyte shortening rates and perhaps the significant increase in maximal Ca^{2+}-activated isometric force observed in this study after PKA treatment. Another possibility for the PKA-induced changes observed in this study but not in others is that myocytes were obtained from young (2- to 3-month-old) male rats that expressed predominantly V_1 myosin isozyme,[25,26] which has been found to be more responsive to β-adrenergic stimulation than preparations containing V_3 myosin.[8,27,28]

As mentioned above, the studies that have investigated β-adrenergic stimulation on cardiac myofilament function have for the most part focused on one or both extremes of the force-velocity relationship (ie, isometric or unloaded contractions). Only a few studies have investigated whether β-adrenergic stimulation affects muscle shortening rates over a broad range of loads. Chiu et al[29] measured the effect of isoproterenol on force-velocity properties of intact rabbit papillary muscles. Isoproterenol increased both shortening velocity and power output at all loads, even when power was normalized for the increase in twitch force associated with β-adrenergic stimulation. However, one limitation of this study is that, because Ca^{2+} activation levels increase after β-adrenergic stimulation, it is impossible to discern the proportion of the increase that resulted from the elevated amount of activator Ca^{2+}[17] versus a direct effect of phosphoproteins on crossbridge cycling rates. The increased power could have arisen entirely from increased $[Ca^{2+}]_i$, especially given the tight Ca^{2+} dependence of myocyte loaded shortening rates.[17] Furthermore, de Tombe and ter Keurs[10] concluded that increased $[Ca^{2+}]_i$ after isoproterenol alone sped unloaded sarcomere shortening of intact trabecular preparations, given

that isoproterenol had no effect on unloaded shortening rates during optimal $[Ca^{2+}]_o$ activations. We attempted to dissect out a phosphoprotein effect from a Ca^{2+} effect per se by utilizing skinned cardiac myocyte preparations, which allowed control of the free Ca^{2+} concentration bathing the myocyte and activating the thin filaments. Thus, our preparation allowed us to examine the direct effects of PKA on crossbridge cycling kinetics during loaded contractions, independently of PKA-mediated increases in myoplasmic Ca^{2+}. The finding that force-velocity relationships were shifted to higher velocities in skinned myocyte preparations after PKA treatment suggests that the previously observed β-adrenergic–induced shifts to faster velocities at similar relative loads in intact cardiac muscle preparations arose, at least in part, from a direct effect of phosphoproteins to speed crossbridge cycling rates. Interestingly, in our study, PKA increased peak normalized power output to a greater extent during submaximal Ca^{2+} activations than during maximal Ca^{2+} activations. This resulted from a greater fractional increase in loaded shortening velocity at F_{opt} (the relative load at which power was optimal) during submaximal Ca^{2+} activations. The exact reasons for this finding are unknown but it suggests that the crossbridge transition(s) that limits peak power output is modulated by phosphoproteins in a Ca^{2+}-dependent manner.

Structural Changes in TnI and MyBP-C After PKA-Induced Phosphorylation

Potential mechanisms for faster myocyte loaded shortening rates after PKA-induced phosphorylation may involve structural changes in either TnI or MyBP-C. As mentioned above, cardiac TnI contains serial serine residues at positions 22 and 23 that are substrates for PKA. Phosphorylation of these residues causes folding of TnI at its N-terminal extension.[30] This conformational change weakens the cooperative binding of TnI to actin-tropomyosin[31] and reduces the affinity of TnI for TnC, especially in the presence of Ca^{2+}.[32,33] Because the interaction between TnI and TnC increases Ca^{2+} binding, reduced TnI/TnC binding lowers the affinity of TnC for Ca^{2+}.[19,22] This reduction in Ca^{2+} binding to TnC after TnI phosphorylation likely underlies the reduced Ca^{2+} sensitivity previously discussed. Moreover, recent evidence from studies in transgenic mice implies that phosphorylation of the two N-terminal TnI residues is necessary to speed maximal velocity of shortening,[14] isometric crossbridge kinetics, and myofibrillar relaxation rates.[24] Thus, it is conceivable that faster crossbridge cycling rates after phosphorylation of TnI may be solely responsible for the increased power observed in this study. Alternatively, the cardiac isoform of MyBP-C is also phosphorylated by PKA after β-adrenergic stimulation. MyBP-C is *tris*-phosphorylated by PKA at a conserved N-terminal domain known as the MyBP-C motif.[34] Phosphorylation of MyBP-C has been reported to relieve the binding of the MyBP-C to the S2 portion of myosin,[35] which may allow greater crossbridge extension from the thick filament and alter crossbridge orientation.[28] These alterations in crossbridge constraints after MyBP-C phosphorylation may allow additional recruitment of crossbridges to bind actin and/or alter crossbridge cycling rates. Future studies are needed to determine precisely which phosphorylation sites on TnI, MyBP-C, or both are most important in conferring increased power generating capacity in cardiac myocytes.

Acknowledgments

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