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Molecule specific effects of PKA-mediated phosphorylation on rat isolated heart and cardiac myofibrillar function

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ABSTRACT

Increased cardiac myocyte contractility by the β-adrenergic system is an important mechanism to elevate cardiac output to meet hemodynamic demands and this process is depressed in failing hearts. While increased contractility involves augmented myoplasmic calcium transients, the myofilaments also adapt to boost the transduction of the calcium signal. Accordingly, ventricular contractility was found to be tightly correlated with PKA-mediated phosphorylation of two myofibrillar proteins, cardiac myosin binding protein-C (cMyBP-C) and cardiac troponin I (cTnI), implicating these two proteins as important transducers of hemodynamics to the cardiac sarcomere. Consistent with this, we have previously found that phosphorylation of myofilament proteins by PKA (a downstream signaling molecule of the beta-adrenergic system) increased force, slowed force development rates, sped loaded shortening, and increased power output in rat skinned cardiac myocyte preparations. Here, we sought to define molecule-specific mechanisms by which PKA-mediated phosphorylation regulates these contractile properties. Regarding cTnI, the incorporation of thin filaments with unphosphorylated cTnI decreased isometric force production and these changes were reversed by PKA-mediated phosphorylation in skinned cardiac myocytes. Further, incorporation of unphosphorylated cTnI sped rates of force development, which suggests less cooperative thin filament activation and reduced recruitment of non-cycling cross-bridges into the pool of cycling cross-bridges, a process that would tend to depress both myocyte force and power. Regarding MyBP-C, PKA treatment of slow-twitch skeletal muscle fibers caused phosphorylation of MyBP-C (but not slow skeletal TnI (ssTnI)) and yielded faster loaded shortening velocity and ~30% increase in power output. These results add novel insight into the molecular specificity by which the β-adrenergic system regulates myofibrillar contractility and how attenuation of PKA-induced phosphorylation of cMyBP-C and cTnI may contribute to ventricular pump failure.

1. Introduction

The mammalian heart has an astonishing capability to vary its pumping capacity from second-to-second. The heart alters ventricular stroke output by fluctuating both physical and activation factors in each individual cardiac myocyte. For instance, increased ventricular filling yields more optimal myofilament lattice properties [9] that increase the propensity for myosin cross-bridges to transition from non-force generating states to force generating states. In addition, ligand binding to beta$_1$ (β_1)-adrenergic receptors increases the intracellular calcium transient $[Ca^{2+}]_i$ [12], which also increases the probability of force generating myosin cross-bridges. The increase in $[Ca^{2+}]_i$ by β_1-adrenergic stimulation is mediated by 3′-5′ cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA)-mediated phosphorylation of calcium handling proteins including the sarcolemmal L-type Ca^{2+} channel, the Ca^{2+} release channel (ryanodine receptor) in the sarcoplasmic reticulum (SR), and the SR protein, phospholamban [3]. In addition, PKA has multiple substrates within the myofilaments including titin [71], the thick filament protein cardiac myosin binding protein-C (cMyBP-C) [8,19,20,58], and the thin filament protein cardiac troponin I (cTnI) [60,61,64]. Thus, β_1-adrenergic stimulation launches a highly coordinated, diverse array of post-translational modifications (PTMs) of calcium handling proteins and

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myofilament proteins, all of which precisely interact to optimize ventricular pump function. One potential interface molecule between augmented $[Ca^{2+}]_i$ and myofibrillar function is cTnI. Phosphorylation of cTnI at serines 23/24 is known to reduce the affinity of cardiac troponin C (cTnC) for Ca^{2+}; this likely assists myofilament deactivation, which is especially important given the elevated $[Ca^{2+}]_i$ transient and the higher heart rates (and the consequent diminished diastolic time interval) due to β_1-adrenergic stimulation. This mechanism would help retain adequate diastolic filling and keep cardiac myocytes working at ideal lengths (i.e., physical environment) during each heartbeat. While there is overwhelming evidence in support of this mechanism i.e., decreased Ca^{2+} sensitivity of force in response to PKA mediated phosphorylation of cTnI [10,30,33–35,56,63,67]), we consider it to be only one of several myofilament alterations elicited by PKA-mediated phosphorylation that adjust ventricular performance to meet hemodynamic demand. Consistent with this, we have found that PKA treatment of permeabilized rat cardiac myocyte preparations not only decreases Ca^{2+} sensitivity of isometric force [24,26,27,30], it also (i) increases maximal Ca^{2+}-activated force [30], (ii) decreases the rate of force development (which we theorize to result from enhanced recruitment of cross-bridges [26,27], (iii) increases both maximal and half-maximal Ca^{2+}-activated power output [27,30], (iv) increases shortening-induced cooperative deactivation [44,45] and (v) augments length dependence of force generation [24,26]. These results have been consolidated into our working model whereby PKA-mediated phosphorylation of myofilament proteins augments contractility by increased cooperative activation of the thin filament following Ca^{2+} binding to cTnC and enhanced cooperative deactivation of the thin filament upon myocyte shortening to help assist with myocyte/ventricular relaxation [24–26,39,44]. If this model, which was derived, for the most part, from biophysical experiments on rat skinned cardiac myocytes, is correct then rat ventricular contractility should correlate with PKA-mediated phosphorylation of myofibrillar proteins. Thus, we hypothesized that rat left ventricular power output at any given pre-load will increase as a function of either PKA-mediated cMyBP-C or cTnI phosphorylation levels or both.

Next, since PKA has multiple myofibrillar substrates (i.e., titin, cMyBP-C, and cTnI) we attempted to define molecular-specificity of functional changes induced by PKA-mediated post-translational modifications (PTMs). For these experiments, we returned to rat skinned cardiac myocyte or slow-twitch skeletal muscle fiber preparations and utilized a troponin complex exchange protocol to help isolate PTM molecule specificity in the control of three key determinants of ventricular stroke performance, i.e., force, rate of force development, and power output.

2. Material and methods

2.1. Experimental animals

All procedures involving animal use were performed according to the Animal Care and Use Committee of the University of Missouri. Male Sprague-Dawley rats (6 weeks of age) were obtained from Harlan (Madison,WI), housed in groups of two, and provided access to food and water ad libitum. A group of rats were treated with propranolol for 7 days by adding 50 mg to 1 L of H_20 and age matched with control rats.

2.2. Solutions

Perfusion buffer for whole heart experiments contained the following (in mmol/L): 118 NaCl, 4.7 KCl, 2.25 $CaCl_2$, 1.2 $MgSO_4$, 1.2 H_2PO_4, 25 $NaHCO_3$, 0.5 Na-EDTA, 11 glucose, 0.4 octanoic acid, 1 pyruvate; plus 0.1% bovine serum albumin (dialyzed against 40–50 vol of the preceding buffer salt solution).

2.3. Whole heart cannulation

Hearts were removed and the aorta was cannulated and perfused with oxygenated perfusion buffer for 10 min in a Langendorff apparatus. The pulmonary vein was then cannulated, and hearts were switched to a working heart system at the perfusate temperature that was set at 34 °C as previously reported [38,57]. Heart rate, blood pressure, aortic flow, and coronary flow were measured at varied preloads both before and (in some preparations) after administration of 0.1 mM epinephrine. Afterload was kept constant at ~80 cm H_2O throughout the experiments. The preload protocol was 3, 5, 7.5, 10, and 15 (cm H_20) to characterize ventricular function curves.

2.4. Recombinant troponin

Rat cardiac troponin C (cTnC), troponin I (cTnI), and troponin T (cTnT) cDNA was isolated as previously described [37]. cDNA encoding the adult *myc*-tagged rat cTnT was generated by PCR addition of an N-terminal *myc*-tag (MMEQKLISEEDL) prior to Ser-2. The individual recombinant rat cTn subunits were expressed in *Escherichia coli* and purified to homogeneity as previously described for the human cTn subunits [50,51]. Recombinant (R) troponin complex used for exchange contained adult rat cTnC, cTnI, and cTnT with an N-terminal *myc*-tag.

2.5. Cardiac myocyte and skeletal muscle fiber preparations

Myocytes were obtained by mechanical disruption of rat hearts as previously described [43]. Skeletal muscle fibers were obtained from Sprague-Dawley rats anesthetized by inhalation of isoflurane (20% (vol/vol) in olive oil) and slow-twitch skeletal muscle fibers were obtained from the soleus muscle as previously described [43].

2.6. Experimental apparatus

The experimental apparatus for mechanical measurements of myocyte preparations and skeletal muscle fibers was the same as previously described [43,46]. Prior to mechanical measurements the experimental apparatus was mounted on the stage of an inverted microscope (model IX-70, Olympus Instrument Co., Japan), which was placed upon a pneumatic vibration isolation table. Mechanical measurements were performed using a capacitance-gauge transducer (Model 403-sensitivity of 20 mV/mg (plus a 10× amplifier for cardiac myocytes) and resonant frequency of 600 Hz; Aurora Scientific, Inc., Aurora, ON, Canada). Length changes were introduced using a DC torque motor (model 308, Aurora Scientific, Inc.) driven by voltage commands from a personal computer via a 12- or 16-bit D/A converter (AT-MIO-16E-1, National Instruments Corp., Austin, TX, USA). Force and length signals were digitized at 1 kHz and stored on a personal computer using LabView for Windows (National Instruments Corp.). Sarcomere length was monitored simultaneous with force and length measurements using IonOptix SarcLen system (IonOptix, Milton, MA), which used a fast Fourier transform algorithm of the video image of the myocyte.

2.7. Solutions

Compositions of relaxing and activating solutions used in mechanical measurements were as follows: 7 mM EGTA, 1 mM free Mg^{2+}, 20 mM imidazole, 4 mM MgATP, 14.5 mM creatine phosphate,

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