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What role does phosphorylation of cardiac troponin I play in elevating cardiac contractility following adrenergic stimulation?

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Myocardial performance is enhanced when adrenergic receptors are stimulated by catecholamines. The enhanced performance is mediated by cAMP dependent protein kinase A (PKA), which phosphorylates several proteins within the cardiac myocyte including cardiac troponin I (cTnI) and myosin binding protein-C (MyBP-C). Phosphorylation of these two proteins by PKA appears to mediate inotropic effects of adrenergic stimulation by directly modulating the rate of cross-bridge cycling. For instance, phosphorylation of cTnI and MyBP-C by PKA increased the power generating capacity of single permeabilized cardiac myocyte preparations (Herron, Korte, McDonald *Circ Res* 89;1184-1190:2001). It is unknown whether phosphorylation of cTnI or MyBP-C alone or phosphorylation of both proteins is necessary to increase power. The purpose of this study was to develop a methodology to test the relative importance of the two PKA phosphorylation sites on cTnI (serine 23 and 24) in mediating the PKA induced increase in myocyte power output. For these experiments, serines 23 and 24 of rat cTnI cDNA were mutated to alanines using site directed mutagenesis. Next, mutated cTnI (cTnI23/24) was expressed in *E. coli* bacteria and purified using ion exchange chromatography. Mutated cTnI (cTnI23/24) was then complexed with purified cTnT and cTnI and this whole troponin (cTn) complex was exchanged for endogenous cTn in permeabilized cardiac myocytes overnight using a cTn exchange buffer (20 mM Imidazole, 200 mM KCl, 5 mM EGTA, 5 mM MgCl₂, 1 mM DTT). The extent of cTn exchange was assessed by quantifying the amount of PKA-induced phosphate incorporation. We observed only a partial reduction in PKA-induced phosphate incorporation following exchange of cTnI23/24, implicating only a partial Tn exchange using these conditions. We are currently seeking to increase the extent of cTnI23/24 exchange after which individual myocyte preparations will be mounted between a force transducer and position motor and myocyte power output generating capacity will be measured before and after PKA induced phosphorylation of myofilament proteins. These experiments will directly assess whether phosphorylation of cTnI by PKA is necessary to elevate myocyte power output.