S23D/S24D, S23A/S24A Mutations in Rat Cardiac Troponin I Depress Myofilament Ca^{2+} Sensitivity, Maximum Tension and ATPase Activity in Reconstituted Rat Cardiac Muscle Fibers.

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Cardiac troponin I (cTnI) is a key subunit of the troponin complex that is involved in the Ca^{2+}-dependent regulation of cardiac muscle contraction and relaxation. The function of cTnI is altered by Protein Kinase A (PKA)-induced phosphorylation of two key serine (Ser) residues at position 23 and 24. PKA-dependent phosphorylation of Ser 23/24 in cTnI results in decreased myofilament Ca^{2+} sensitivity and accelerated rate of cardiac muscle relaxation. Because phosphorylation induces negative charges at Ser 23/24, recombinant mutant approaches have been used in which Ser 23/24 were mutated to the negatively charged amino acids. For example, aspartic acid has been used to create cTnI S23D/S24D to mimic the phosphorylated state of cTnI, whereas alanine has been used to create cTnI S23A/S24A to mimic a non-phosphorylated state of cTnI [Dohet et al., 1995]. These studies have shown that reconstitution of mutant human cTnI (cTnI S23D/S24D) into porcine cardiac fibers decreases Ca^{2+}-activated force and myofilament Ca^{2+} sensitivity. However contradictory observations have been reported by others, which may have resulted from the use of heterologous proteins. To better understand the impact of mutations at Ser 23/24, we used a homologous system in which rat cTnI mutants were reconstituted into rat cardiac fibers. We measured Ca^{2+}-activated tension and ATPase activity in detergent-skinned rat cardiac fibers reconstituted with mutant rat cTnI constructs, cTnI S23D/S24D or cTnI S23A/S24A. Our study shows that, in addition to a significant decrease in myofilament Ca^{2+} sensitivity, the maximum Ca^{2+}-activated tension and ATPase activity also decrease significantly in fibers reconstituted with cTnI S23D/S24D. Surprisingly, reconstitution with cTnI S23A/S24A also resulted in decreased myofilament Ca^{2+} sensitivity, Ca^{2+}-activated tension and ATPase activity. Thus, our study suggests that structural alterations in cTnI at these key residues suppressed contractile function.