SUMMARY

Cardiac Troponin I Phosphorylation: Exchanging Ideas

In Chapter 1 a general introduction to the background and methods of the studies is given.

In Chapter 2 phosphorylation levels of cTnI and of other myofilament proteins in non-failing donor, ICM and IDCM cardiomyopathy hearts was studied in concert with cardiomyocyte contractility. In addition, protein phosphatase type 2A (PP2A) expression, PP2A activity and the effects of PP2A incubation in donor, ICM and IDCM were studied. cTnI dephosphorylation by PP2A at cTnI-Ser23/24 was studied via western blotting.

In Chapter 3 effects of site-specific pseudo-phosphorylation of cTnI at the PKA sites Ser23 and Ser24 are described in human cardiomyocytes. Four different cTn complexes were made by site-directed mutagenesis of cTnI at Ser23/24: 23A/24A, 23A/24D, 23D/24A, 23D/24D. These recombinant cTn complexes were exchanged in donor cardiomyocytes. In addition, the effect of bisphosphorylation on Ca^{2+}-sensitivity was titrated in IDCM cardiomyocytes.

In Chapter 4 PKC-mediated effects of cTnI phosphorylation at Thr143 on LDA of the myofilament was studied. Therefore, human wild-type cTn complex and cTn complex pseudo-(de)phosphorylated at Thr143 was exchanged in non-failing donor and in IDCM cardiomyocytes. Subsequently, force was measured at short (1.8 µm) and long (2.2 µm) sarcomere lengths. Additionally, combined effects of PKA-mediated phosphorylation and Thr143 pseudo-phosphorylation on myofilament LDA in donor cardiomyocytes were studied.

In Chapter 5 functional effects of PKC-mediated phosphorylation of Ser42/44 are described. Recombinant wild-type cTn and cTn pseudo-(de)phosphorylated at Ser42/44 was exchanged in IDCM and donor cardiomyocytes. Effects of pseudo-phosphorylation on force development were determined. In addition, combined effects of PKA-mediated phosphorylation and Ser42/44 pseudo-phosphorylation on myofilament LDA were studied. ATPase activity was measured in cTn-exchanged muscle strips.

In Chapter 6 phosphorylation of cTnI at a recently identified PKC-mediated phosphorylation site was studied, Ser199. By exchanging recombinant wild-type cTn and cTn pseudo-(de) phosphorylated at Ser199 in IDCM and donor cardiomyocytes, effects on force development were studied. In addition, the effect of Ser199 phosphorylation on Ca^{2+}-sensitivity was titrated in IDCM and donor cardiomyocytes.

In Chapter 7 we investigated whether high myofilament Ca^{2+}-sensitivity and perturbed LDA is characteristic for human HCM with mutations in thick and thin filament proteins. Therefore, cardiac samples from HCM patients harboring mutations in genes encoding thick (*MYH7, MYBPC3*) and thin (*TNNT2, TNNI3, TPM1*) filament proteins were compared with sarcomere mutation-negative HCM and non-failing donors. LDA was studied in patients with mutations in *TNNT2* and *TNNI3* after replacement of mutated troponin with wild-type troponin.

In Chapter 8 a summary and conclusion of the major findings of the studies presented in this thesis is given followed by future perspectives.