Contractile Function of the Human Myocardium

Impact of Troponin Phosphorylation

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Cover: Schematic representation of the cardiac thin filament

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Conclusions & Future perspectives

In present thesis, the effects of phosphorylation of contractile proteins on force development in healthy and failing cardiac tissue were investigated with a focus on PKA- and PKC-mediated phosphorylation. Both beneficial as well as detrimental effects on force development of the cardiomyocytes have been found and described in this thesis. The troponin exchange method used provided insight into the specific effects of PKA-and PKC-mediated phosphorylation of cTn on contractile properties of single human cardiomyocytes. This technique allowed separation of the specific effects of cTn phosphorylation and phosphorylation of the other myofilament proteins (the phosphorylation background).

The main findings and conclusions of this thesis

I. Direct incubation of single cardiomyocytes with PKCα and PKCε reduced Ca^{2+}-sensitivity in human failing cardiomyocytes via phosphorylation of cTn and/or cMyBP-C. This was not apparent in donor cardiomyocytes presumably because the phosphorylation levels were already very high. The reduction in Ca^{2+}-sensitivity is considered to be beneficial for relaxation of the heart (Chapter 2).

II. Targeted phosphorylation of cTn with PKCα using a cTn exchange method resulted in a sensitisation of failing cardiomyocytes to Ca^{2+}. In addition, subsequent incubation of the cardiomyocytes with PKCα decreased the Ca^{2+}-sensitivity as observed in Chapter 2. Based on these findings, we argue that the effects of PKCα-mediated phosphorylation on the Ca^{2+}-sensitivity are complex. The results suggest that the *in vivo* outcome of PKCα-mediated phosphorylation depends on the phosphorylation status of the target proteins at the time of receptor activation.

III. PKCα-targeted phosphorylation of cTn resulted in a significant reduction of the maximal force generating capacity of human cardiomyocytes. This effect could not be corrected by additional incubation of the cardiomyocytes with PKCα. Based on these results we conclude that PKCα-mediated phosphorylation of cTnI and/ or cTnT is able to decrease the maximal force generating capacity of the cardiomyocytes (Chapter 3).

IV. Liquid chromatography (LC) MS/MS analysis of recombinant cTn complex incubated with PKCα revealed two 'novel' phosphorylation sites. Ser199 located on cTnI and Ser179 on cTnT were identified as PKCα substrates (Chapter 3). Phosphorylation at the newly identified PKC sites in human troponin may contribute to the intricate effects of PKCα on myofilament function.

V. Using the cTn exchange method, we observed that cTnI phosphorylation at the PKA sites in failing cardiomyocytes did not decrease the Ca^{2+}-sensitivity. However, direct incubation of failing cardiomyocytes with PKA resulted in a desensitisation of the myofilaments to Ca^{2+}. From these results we suggest that the sarcomeric phosphorylation background, which is altered during cardiac disease, influences the impact of PKA-mediated cTnI Ser23/24 phosphorylation on Ca^{2+}-sensitivity (Chapter 4).

VI. Pseudo-phosphorylation of Ser23/24 on cTnI by replacement with aspartic acid does not completely mimic the physiological effects of PKA-mediated bis-phosporylation on force development at submaximal Ca^{2+}-concentrations (Chapter 5).

Future perspectives

Heart failure is worldwide an enormous social and economical problem due to high morbidity and mortality rates. Consequently, both prevention and treatment of the disease is of great importance and priority. Phosphorylation has been shown to be an important determinant of cardiac performance. It could, therefore, be a potentially effective target for therapy. However, more research is needed to increase our understanding about the effects of the individual phosphorylation sites and how they eventually can be used as a therapeutical target or as diagnostic indicators.

- Future reseach should focus on the effects of the individual phosphorylation sites, other than the PKA-sites, in human cardiac tissue. The individual phosphorylation sites have diverse effects on the contractile properties of the cardiomyocytes and might even influence the effects of each other. Site-directed mutagenesis could be used to mimic phosphorylation of the known sites on cTnI and cTnT. As performed in the current study, the experiments should be conducted in both failing and donor tissue and even in tissue obtained during cardiac catheterization. In this way, the (possibly diverse) effects in a healthy and diseased phosphorylation background can be observed.

- It has been generally accepted that phosphorylation of cTnI by PKA decreases the Ca^{2+}-sensitivity of force generation, which is considered to be beneficial for the diastolic phase of the contraction cycle of the heart [31,106]. As a consequence, cTnI phosphorylation might be suitable as therapeutical target. However, results in this thesis show that targeted phosphorylation of Ser23/24 on cTnI does not lead to a desensitisation of the myofilaments

to Ca^{2+} in end-stage failing cardiomyocytes. This discrepancy in the results might have been caused by a difference in background phosphorylation. In our study using targeted phosphorylation of cTnI, the phosphorylation status of other PKA substrate proteins (e.g. cMyBP-C, titin) was not modified.

PKA has more targets besides myofilament proteins; ryanodine receptors, L-type Ca^{2+}-channels and phospholamban. Activation of PKA as therapeutic intervention will result in an overall phosphorylation of PKA substrates (e.g. myofilament, sarcolemmal and membrane proteins). Consequently, the additive effects will determine clinical outcome and could be either detrimental or beneficial. A more effective way to target specific phosphorylation of Ser23/24 on cTnI could be the use of pseudo-phosphorylated cTnI. Results in this thesis show that replacement of Ser23 and 24 with aspartic acid desensitised the myofilaments to Ca^{2+}. Our results indicated that the influence of the phosphorylation 'background', as observed before, might have been abolished with pseudo-phosphorylated cTn complex. Future research could focus on the use of cTn(DD) complex as therapeutical target to improve the diastolic phase of the cardiac cycle. For example, in combination with viral gene transfer it might provide a strategy to tailor specific physiological outcomes in the heart

- We believe that cTnI phosphorylation status changes with phenotype and stage of the disease and that these changes reflect the severity of the disease [23]. Consequently, altered phosphorylation levels at specific cTn sites will provide increased understanding and allow targeting of specific sites for therapy. Generally, one third of the patients with a myocardial infarction end up with heart failure within 1-2 years. However, currently there is no test to determine which patients are at risk of heart failure. Therefore a diagnostic assay based on the quantitative analysis of the phosphorylation sites on cTn could be used for diagnosis, prognosis or risk stratification in patients with cardiac disease. More research is necessary to explore the use of cTn phosphorylation as diagnostic marker.