Chapter 6

**AKIP1 promotes physiological hypertrophy after voluntary exercise**

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*under progress*
Chapter 6

Abstract

Background
Overexpression of A Kinase Interacting Protein 1 (AKIP1) promotes physiological hypertrophy in cultured cardiomyocytes. Whether AKIP1 stimulates physiological cardiac hypertrophy in vivo is unknown.

Methods and results
Mice with cardiomyocyte-specific overexpression of AKIP1 (AKIP1-TG) and their wild type (WT) littermates were subjected to 4 weeks of voluntary wheel running, whereas control mice remained sedentary. While running time and distance were comparable between AKIP1-TG and WT mice, Heart weight / tibia length was markedly increased in AKIP1-TG mice after voluntary exercise (9.5± 0.3 mg/mm in AKIP1-TG vs. 8.7± 0.2 mg/mm in WT mice, p<0.05). The augmentation of cardiac hypertrophy was associated with a 6-fold increase in AKT-phosphorylation upon exercise.

Conclusion
Cardiomyocyte-specific overexpression of AKIP1 promotes physiological cardiac hypertrophy after voluntary exercise.
Introduction

Heart failure (HF) is a huge burden on the health of many people. It is expected that one third of the inhabitants of the developed world will develop HF in their lifetime.\textsuperscript{1,2} Mortality in HF is worse than in most types of malignancies.\textsuperscript{3} To reduce this burden, we need interventions that can prevent HF or attenuate the progression of HF.

When the heart is faced with physiological and pathophysiological stress, cardiac hypertrophy develops. Hypertrophy is an adaptive mechanism that reduces wall stress and allows the heart to adapt to the increased workload. Indeed, cardiac hypertrophy that develops upon exercise or during pregnancy augments cardiac performance and is fully reversible. During sustained pathological stress, compensatory hypertrophy progresses into a pathological phenotype.\textsuperscript{4,5} The differences in physiological versus pathological hypertrophy are recapitulated by the activation of very distinct signal transduction pathways and transcription factors. Pathological hypertrophy is characterized by fibrosis, apoptosis and fetal gene expression whereas these features are not observed in the physiological setting.\textsuperscript{6} One strategy to alleviate pathologic cardiac remodeling is to superimpose the beneficial aspects of physiological hypertrophy upon pathologically remodeled hearts. Indeed, pathological hypertrophy can be reversed by physiological exercise in experimental HF models.\textsuperscript{7,8} Furthermore, it has been shown that exercise training attenuates cardiovascular mortality and HF hospitalizations in HF patients.\textsuperscript{9}

We previously performed a screen of several \textit{in vivo} and \textit{in vitro} models of cardiac hypertrophy and HF to determine putative molecular targets for therapy. One of the genes consistently upregulated in these models was A Kinase Interacting Protein 1 (AKIP1).\textsuperscript{10} Several gain of function experiments demonstrated that AKIP1 could induce a physiological type of hypertrophy in cultured cardiomyocytes as evidenced by the improved mitochondrial respiration and a lack of fetal gene expression.\textsuperscript{11} Surprisingly, cardiomyocyte-specific overexpression of AKIP1 did not cause spontaneous cardiac hypertrophy \textit{in vivo}.\textsuperscript{12} Also, development of pathological hypertrophy and HF remodeling was not affected by AKIP1 after pressure overload or myocardial infarction, suggesting that AKIP1 does not regulated cardiac growth in heart disease. In summary, several lines of evidence suggest that AKIP1 is a specific regulator of physiological hypertrophy. To investigate this hypothesis, AKIP1-TG mice were subjected to voluntary wheel running to determine the effect on cardiac function and hypertrophy.
Methods

Animals and experimental model
AKIP1-transgenic mice (AKIP1-TG) were created as previously described. These mice are phenotypically normal and have a reproduction pattern with normal mendelian ratios. AKIP1-TG mice and wild type (WT) littersmates of 8-12 weeks of age were individually housed with unlimited access to a running wheel for the duration of 4 weeks. Control mice were housed similarly but in the absence of a running wheel. Running distance and running time were measured daily with a cyclometer connected to the running wheel. After 4 weeks, cardiac function was determined with MRI as previously described.

Left ventricular mass, left ventricular end-systolic and end-diastolic dimensions were quantified with Qmass (version MR 6.1.5, Medis Medical Imaging Systems, The Netherlands). Finally, mice were anesthetized and intra-cardiac pressures were measured with a pressure volume system (ADVantage Admittance PV system, Transonic Scisense Inc, London, ON, Canada) and analyzed with Labchart 7 (ADInstrument Ltd, Dunedin, New Zealand). Hereafter, hearts were excised. Left ventricle, right ventricle and atria were separated, weighed and snap frozen in liquid nitrogen for molecular analysis.

Western Blot
Western blot was performed as described previously. An AKIP1 antibody was made in our lab as described previously. Antibodies for anti-phosphorylated-AKT\textsuperscript{Ser473} and anti-total-AKT (cell signaling) were bought commercially.

Statistical analysis.
Values are displayed as mean ± standard error of the mean. Comparisons were made with Student \( t \) test or one-way ANOVA followed by the Tukey post-hoc test where appropriate. A p-value <0.05 was considered significant.

Results
To investigate the effect of AKIP1-overexpression on development of physiological hypertrophy of the heart, we used transgenic mice with cardiac specific over-expression of AKIP1 as previously described. AKIP1-TG mice and WT littersmates were subjected to a regimen of 4 weeks of voluntary exercise or a sedentary control group. During the first week, running distance for both groups increased and then remained stable for the remainder of the experiments (Figure 1A). Running time picked up in the first few days and remained stable over the course of the 4 weeks (Figure 1B). Average daily running distance, running time and average speed were similar between both genotypes in the exercise groups (Figure 1A,B,C). Voluntary exercise resulted in a marked increase in heart weight and left ventricular weight in AKIP1-TG
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Figure 1 Running distance and heart weight after 4 weeks voluntary wheel running. Graphs depict the running distance (A), running time (B) and average running speed (C). Line graphs represent the mean ± SE for each day and bar graphs represent the average over 4 weeks with mean ± SE. N=9-12 animals for the groups. Statistical testing was performed with a Student t test; n.s., not significant. (D) Cardiac overexpression of AKIP1 increases heart weight and left ventricular weight corrected for tibia length after 4 weeks voluntary wheel running. Heart weight, HW; LV, Left Ventricular weight; TL; Tibia length. N=9-21 animals per group. Error bars represent mean ± SE. Statistical testing was performed by one-way ANOVA; *, p<0.05; **, p<0.01; n.s. not significant.

mice compared to their WT littermates (Figure 1D).

To evaluate whether the differences in cardiac weight translated into differences in cardiac function, we performed cardiac MRI and pressure-volume measurements at the end of the study. As observed before, left ventricular ejection fraction was slightly reduced in sedentary AKIP1-TG animals compared to the WT littermates (Figure 2A). This was not altered by exercise. We observed a trend to increased left ventricular end diastolic mass as determined by MRI, but this was not significant (Figure 2A). Left ventricular end-diastolic volume was similar between the experimental groups (Figure 2A). Left ventricular end-systolic volume was increased in sedentary AKIP1-TG compared to WT mice.

Indices of cardiac contractility (dP/dt max) and relaxation (dP/dt min, Tau) were determined by pressure-volume loop analysis before sacrifice. There
Figure 2 Overexpression of AKIP1 does not influence cardiac remodeling after transverse aortic constriction (TAC). (A) Cardiac function parameters as determined by magnetic resonance imaging and representative end-diastolic images of AKIP1-transgenic (AKIP1) and wild-type (WT) mice. Shown are Left Ventricular End-Diastolic Mass, LVEDmass corrected for Tibia Length, TL; Left Ventricular Ejection Fraction, LVEF; Left Ventricular End-Diastolic Volume, LVEDV and Left Ventricular End-Systolic Volume, LVESV. N=9-21 animals per group. Error bars represent mean ± SE. Statistical testing was performed by one-way ANOVA; *, p<0.05; ***, p<0.001; n.s. not significant. (B) Intracardiac pressure and relaxation measurements. Shown are dP/dtmax, dP/dtmin and Tau. N=6-12 animals per group. Error bars represent mean ± SE. Statistical testing was performed by one-way ANOVA; *, p<0.05; n.s. not significant.

was a slight decrease in dP/dt min in AKIP1-TG vs. WT sedentary mice, but contractility and relaxation measurements were similar between genotypes after 4 weeks of voluntary wheel running (Figure 2B).

The signal transduction molecule AKT is a central driver of physiological hypertrophy. Previous research from our group demonstrated that the pro-hypertrophic effect of AKIP1 in cultured cardiomyocytes was regulated by AKT. Accordingly, we tested whether the salutary effect of AKIP1 overexpression on physiological hypertrophy was associated with activation of AKT. Voluntary wheel running was associated with a significant increase in AKT phosphorylation after exercise. While AKT phosphorylation was comparable between the genotypes in sedentary animals, AKIP1 transgenic mice displayed a significant greater augmentation of AKT phosphorylation than WT animals after exercise (Figure 3).
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**Figure 3** Myocardial AKT-phosphorylation after voluntary wheel running. Shown are densitometric analysis and representative blots. Samples were run on the same gel and pictures were taken from the same blot and same exposure time. \( N=3-4 \) animals per group. Error bars represent mean ± SE. Statistical testing was performed by one-way ANOVA (left panel) or Student \( t \) test (right panel); *, \( p<0.05 \); n.s. not significant.

**Discussion**

In the current study we demonstrate that cardiomyocyte-specific overexpression of AKIP1 results in a marked augmentation of physiological hypertrophy, as evidenced by markedly greater augmentation of heart weight and left ventricular mass than WT mice after voluntary wheel running. This difference could not be explained by differences in the amount or intensity of exercise as these parameters were comparable between genotypes. The stimulation of cardiac hypertrophy by AKIP1 was not accompanied by the development of systolic or diastolic cardiac dysfunction. Furthermore, the augmentation of cardiac hypertrophy in AKIP1-TG mice was associated with a 6-fold greater increase in the activation of AKT. Together, these findings indicate that overexpression of AKIP1 is a central and specific driver of physiological hypertrophy after exercise.

Cardiac hypertrophy is the response to increased cardiac workload and wall stress. Previous research suggests there is a beneficent, physiological type of hypertrophy and a maladaptive type of hypertrophy.\(^4,5\) We previously showed that AKIP1 increased hypertrophy in cultured cardiomyocytes.\(^11\) This hypertrophy occurred in the absence of several transcriptional and phenotypic signatures of pathological hypertrophy. Instead, AKIP1-overexpression was found to activate AKT and several downstream mediators of physiological hypertrophy. Importantly, cardiomyocyte hypertrophy induced by AKIP1 overexpression was blocked by the addition of an AKT-inhibitor. As AKT has been identified as a central regulator of physiological hypertrophy,\(^4\) Our finding that cardiomyocyte-specific overexpression of AKIP1 stimulates cardiac hypertrophy and AKT phosphorylation, confirms our previous findings in cultured cardiomyocytes and reinforces the role for AKIP1 in physiological growth.
We observed that left ventricular ejection fraction was slightly decreased in AKIP1-TG animals. However, this difference was not influenced by voluntary wheel running. It should be noted, however, that our results were measured during rest. In trained human athletes it has been demonstrated that cardiac function is also slightly reduced when in rest, but becomes supraphysiological during exercise.\textsuperscript{13,14} It is tempting to speculate that a similar phenotype exist in AKIP1-TG mice, but it would require additional experiments where cardiac function is measured during physiological or pharmacological exercise.

In the current analysis, we measured cardiac function after 4 weeks voluntary wheel running. We did not determine cardiac function before the experiment and thus could not determine the alterations in cardiac function within each experimental group. Also, this protocol consisted of voluntary exercise. The results in a forced exercise experiment might be different. Furthermore, additional measurements of biochemistry and histological measurements like cell size and fibrosis are required to claim that hypertrophy does indeed have a physiological phenotype.

In conclusion, Cardiomyocyte-specific overexpression of AKIP1 promotes physiological cardiac hypertrophy after voluntary exercise.

References