The effects of CaMKII inhibition on cardiac muscle contractility in the zucker diabetic fatty rat

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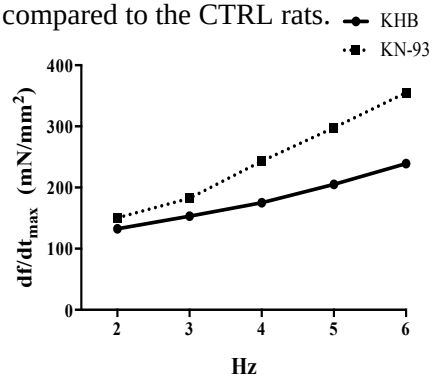
Background. The incidence of diabetes mellitus (DM) is growing rapidly, with 8 million people newly diagnosed globally each year. The landmark Framingham study showed that the incidence of heart failure (HF) is significantly higher in diabetic compared with age-matched controls (CTRL) (Kannel & McGee, 1979). However, the underlying mechanisms that link DM to HF are still unclear. Recent studies show that DM patients and mouse models of diabetes have an up-regulation of calmodulin-dependent protein kinase (CaMKII) activity (Luo *et al.,* 2013). CaMKII is a multifunctional serine-thereonine kinase, which upon activation is involved in coordination of the ion channels and Ca^{2+} handling proteins involved in excitation-contraction and excitation-transcription coupling in the myocardium. Excessive CaMKII activity in the myocardium promotes hypertrophic and apoptopic cardiomyopathy, ultimately leading to HF due to reduced mechanical performance in the myocardium (Anderson *et al.,* 2011). In addition, persistent CaMKII activation has been proposed to underlie arrhythmogenic events in the heart by promoting Ca^{2+} leak from the sarcoplasmic reticulum (Chelu *et al.,* 2009). Oxidative stress can lead to activation of CaMKII, as can hyperglycemia through glycolysation (*O*-GlcNAc) of the kinase (Erickson *et al.,* 2013). Therefore the discovery that CaMKII activity is increased in DM, and that both oxidative stress and hyperglycaemia can also modulate CaMKII activity, suggests a potential signaling role for oxidative stress and *O*-GlcNAc in the diabetic heart. It was hypothesized that inhibition of CaMKII activity will preserve muscle contractility in the zucker diabetic fatty rat (ZDF) heart.

Methods. At 12, 16 and 20 weeks blood glucose was measured and an echocardiography scan for the assessment of cardiac function after the animal had been anesthetized with 3% isoflurane on ZDF (n=10) and CTRL rats (n=10). At 20 weeks rats were killed with an overdose of pentobarbital (60mg/kg) and the heart extracted. It was mounted on a simplified Langendorff setup and perfused with Tyrode with 20mM butanedione monoxime. The right ventricle was opened and a suitable cardiac muscle (trabeculae) dissected. The trabeculae was attached between a force transducer and micromanipulator, immersed in carbogenated Krebs-Henseleit solution (37°C). Measurements taken included: force frequency characteristics, sarcoplasmic reticulum Ca^{2+} load and maximal force in response to isoproterenol ($10^{-8,-7,-6}$). Measurements were then repeated in the presence of inhibitors blocking CaMKII (KN-93, 2µmol/L). CTRL experiments were performed using the inactive analogue KN-92 (2µmol/L). Sections of heart tissue from the right and left ventricle were rapidly snap frozen in liquid nitrogen for later protein analysis of total, oxidized and *O*-GlcNAc CaMKII, or fixed in 4% formalin and later cryostat sectioned for staining of fibrosis (Masson's trichrome) and apoptosis (TUNEL).

Results. After 12 weeks, fasted blood glucose was 17.4mmol/L for the ZDF diabetic rats and 8.6mmol/L for the CTRL rats. No significant differences were detected in heart function as measured by echocardiography at 12 weeks. However, at 16 weeks we observed changes in the E/A ratio in the ZDF rat from 1.77 to 2.19 compared to 1.95 in the CTRL rats, indicating potential functional remodeling, in particular diastolic function, in the diabetic animals. We were able to measure force frequency, SR Ca^{2+} load and maximal force in response to cardiac stress in diabetic and non-diabetic animals, both with and without the CaMKII inhibitor KN-93 (see Figure). Inhibition of CaMKII resulted in improved maximum rate of contraction, as well as reduced relaxation time in the non-diabetic rat heart. Indicating improved contractility and relaxation after CaMKII inhibition. Western blot analysis showed an up regulation of total CaMKII, oxidized, *O*-GlcNAc CaMKII in the ZDF rat compared to the CTRL rats.



Maximum rate of contraction in trabeculae isolated from the right ventricle of non-diabetic animals. KHB: no CaMKII inhibition; KN-93: CaMKII inhibition.

Conclusion. The results from this study show that the diabetic heart is predisposed to decreased cardiac function as diabetes develops, and that total, oxidized, and *O*-GlcNAc modified CaMKII are up regulated in the diabetic heart. The results from our functional studies suggest a potential therapeutic role for CaMKII inhibitors in improving cardiac muscle contractility in the diabetic heart.

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