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199 TAKOTSUBO SYNDROME ASSOCIATED MIR-16 AND MIR-26A REDUCE CONTRACTILITY OF CARDIOMYOCYTES IN VITRO BY AN INHIBITORY G-PROTEIN DEPENDENT MECHANISM

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Introduction Takotsubo syndrome (TTS) is a severe but reversible acute heart failure affecting predominantly post-menopausal women, where ventricular apical akinesis results from extreme adrenaline levels arising with stress. The pleiotropic β^2AR signals via stimulatory (G_s) and inhibitory (G_i) G-proteins, and whilst G_s increases cardiac output, it concomitantly decreases survival. The duality of β^2AR is a homeostatic mechanism to limit cardiotoxicity by facilitating a switch to G_i, serving as cardioprotective despite being cardiodepressive. This is dysregulated in TTS where excess stimulus trafficking to G_i results in profound negative inotropy. It is not understood what predisposes patients to TTS, but a microRNA (miR) profile of increased miR-16 and miR-26a has been identified. Given the importance of miRs in other cardiac diseases and that TTS is thought to be causally related to Î²2AR-G_i, we hypothesise that these miRs could predispose to the cardiodepression in TTS.

Method miRs were manipulated in adult rat apical cardiomyocytes with blinded transfection using Lipofectamine 3000. Percentage shortening was measured using an Ionoptix system, and pharmacological protocols applied. Calcium transients were obtained using Fluo-4-AM and sarcoplasmic reticulum (SR) calcium content measured with caffeine micro-application. N numbers displayed as n/N, where n/N=cells/rats.

Results Up-regulation of miR-16 and miR-26a significantly reduced basal contractility (miR-16=3.52±0.34% versus control=4.91±0.46%; n/N=30/6; $p<0.05$ and miR-26a=2.77 ±0.21% versus control=4.30±0.43%; n/N=50/10; $p<0.01$), whereas down-regulation had no effect. miR-16/-26a manipulation did not alter Î²2AR response. Inhibiting Gi with pertussis toxin (PTX) prevented this (miR-16 untreated=5.08±0.49%, n=22; versus miR-16 PTX-treated=8.82±0.63%, n=20; $p<0.001$; and miR-26a untreated=3.20±0.29% versus miR-26a PTX-treated=5.05±0.48%; n/N=30/6; $p<0.05$ respectively). PTX-treatment did not change contractility of control transfected cells. No synergism was observed with dual miR-16/-26a transfection possibly suggesting a unified mechanism. Calcium transient amplitude was decreased with miR-16/-26a up-regulation (F/F0 for control=1.85±0.04, n/N=66/4 versus miR-16=1.51±0.04, n/N=37/4 and miR-26a=1.57±0.04, n/N=26/4; $p<0.001$), along with a concomitant decrease in SR calcium content (caffeine-induced F/F0 for control=3.13 ±0.14, n/N=32/4 versus miR-16=2.13±0.13, n/N=22/4 and miR-26a=2.57±0.19, n/N=17/4; $p<0.001$ and $p<0.05$ respectively).

Conclusion/implication Increased miR-16/−26a reduce basal contractility of cardiomyocytes *in vitro*, possibly through a shared G_i-dependent mechanism. Decreased calcium transient amplitude is also likely to contribute. This suggests these miRs may be mechanistically involved in TTS, but further work is needed to investigate their specific mechanistic and spatiotemporal involvement.

200 NITRIC OXIDE PROMOTES INSULIN-INDEPENDENT GLUCOSE UPTAKE AND PRESERVES CARDIAC FUNCTION AND ENERGETICS IN DIABETES

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Introduction In the presence of diabetes (DM), myocardial glucose uptake and glycolysis are impaired and the heart rapidly adapts to use exclusively fatty acids (FA) for ATP generation. This maladaptation is believed to play a key role in the development of a cardiomyopathy over time. Here, we show that stimulating myocardial nitric oxide synthase (NOS) activity is sufficient to alleviate myocardial metabolic inflexibility, improve energy metabolism and prevent LV dysfunction in DM by increasing myocardial insulin-independent glucose transport.

Methods Myocardial-specific overexpression of GTP cyclohydrolase I (mGCH1) was used to increase both tetrahydrobiopterin (BH4) and NOS activity in cardiomyocytes. Diabetes mellitus (DM) was induced by multiple low-dose streptozotocin injections (vs sham). PCr/ATP ratio was measured in perfused hearts using ^{31}P-MRS, glucose transport estimated by deoxy-glucose uptake, and oxygen consumption rate (OCR) of intact cardiomyocytes using a phosphorescent probe.

Results As expected, sham-injected mGCH1 transgenic hearts had higher BH4 levels and constitutive NOS activity compared with WT. 12 weeks after DM induction, LV dysfunction developed in WT mice but not in mGCH1 mice, in the absence of changes in myocardial BH4 content and NOS activity in either group. WT diabetic hearts had a lower PCr/ATP ratio (1.32±0.1 vs 1.73±0.1, $p<0.05$, n=11 per group) and mitochondrial creatine kinase (CK) activity (1.56±0.1 AU vs 1.98 ±0.1 AU, $p<0.005$, n=10 per group) when compared with non-diabetic WT mice, consistent with impaired cardiac energetics. By contrast, PCr/ATP and CK activity were preserved in diabetic mGCH1 hearts in the absence of differences in myocardial mitochondrial content.

Myocardial GCH1 overexpression was associated with a higher protein levels of the insulin-independent glucose transporter, GLUT-1 ($p<0.05$, n=12 per group), but no changes in GLUT-4 protein. Myocardial glucose transport was 40% higher in LV myocytes from mGCH1 diabetic mice when compared with WT diabetic mice. This was accompanied by increased myocardial glucose oxidation, as determined by OCR. Pre-incubation of myocytes with inhibitors of NOS-PKG signalling (L-NAME, 1 mmol/L or Rp8pCPT PET cGMP 10 µmol/L) or GLUT-1 (STF-31, 10 µmol/L,) abolished all differences between mGCH and WT diabetic hearts.

Conclusions Our study reveals that a myocardial increase in BH4 and NOS activity is sufficient to maintain a favourable substrate utilisation and preserve cardiac mitochondrial function in the presence of DM. This work provides new insight into the potential metabolic triggers of diabetic