

duction. Morphological assessment using electron microscope revealed that mitochondria in the hearts of STZ-induced diabetic rats were swollen and the structure of cristae was collapsed; however, these changes were attenuated by the treatment with KN-93 and apocynin. This observation indicates that activation of CaMKII followed by NADPH oxidase-derived ROS increase contributes to mitochondrial dysfunction which may lead to cell death.

Conclusion: Increase in NADPH oxidase by activating CaMKII is a mechanism of ROS increase in the heart of diabetes mellitus. Up-regulation of ROS production facilitates mitochondrial malfunction and may result in cardiac dysfunction in diabetic patients.

239 The soluble guanylate cyclase activator cinaciguat improves cardiac dysfunction in diabetes mellitus



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Purpose: Patients with diabetes mellitus exhibit cardiovascular dysfunction along with increased oxidative stress and decreased nitric oxide – cyclic guanosine monophosphate (cGMP) signalling. It has been reported, that elevated intracellular cGMP-levels contribute to an effective cytoprotection against oxidative stress. In this study we investigated the effects of cinaciguat, a newly developed soluble guanylate cyclase activator on myocardial dysfunction in type-1 diabetic rats.

Methods: In male Sprague-Dawley rats diabetes was induced by a single ip. injection of streptozotocin (60mg/kg). In the treatment groups, cinaciguat (10mg/kg/d) was applied orally for 8 weeks. Rats of the control groups received vehicle for the same time. After the treatment left ventricular (LV) pressure-volume relations were measured by using a microtip Millar pressure-volume conductance catheter, and indexes of contractility (e.g. preload recruitable stroke work (PRSW)) were calculated. Blood plasma and myocardial tissue samples were collected for determination of cGMP-levels and immunohistochemical analysis, respectively. Myocardial gene expression analysis has been performed by quantitative real-time polymerase chain reaction (qRT-PCR).

Results: When compared to the non-diabetic controls, diabetic rats showed impaired left ventricular contractility (PRSW: 49.5±3.3 vs. 83.0±5.5mmHg; p<0.05) and a marked diastolic dysfunction (time constant of LV pressure decay, Tau: 17.3±0.8 vs. 10.3±0.3ms), which was significantly improved by cinaciguat (PRSW: 66.8±3.6mmHg, Tau: 14.9±0.6ms in the diabetes+cinaciguat group). The treatment had no hemodynamic effects in non-diabetic control animals. Diabetes was associated with increased myocardial immunoreactivity for transforming growth factor beta (TGF-β) along with enhanced gene expression for atrial natriuretic peptide (ANP), heat shock protein 70 (Hsp70) and matrix metalloproteinase-9 (MMP-9), which were all reversed after cinaciguat treatment. Efficacy of the therapy was reflected by markedly elevated levels of cGMP in the diabetes+cinaciguat group compared to control (44.2±11.4 vs. 16.1±2.0pmol/ml plasma).

Conclusions: Our results demonstrate that cinaciguat prevents diabetes-associated deleterious myocardial changes and improves diabetic cardiac dysfunction in our rat model. Pharmacological soluble guanylate cyclase activation might represent a novel therapy approach for diabetic cardiomyopathy.

240 Cx43 hemichannels of the inner mitochondrial membrane are constitutively active in heart failure



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Background: Ischemic preconditioning involves activation of multiple signalling pathways with mitochondrial ion channels as an end-effector. Recently, we demonstrated that connexin 43 hemichannels found in the inner mitochondrial membrane (mito-Cx43 hemi) are essential for cytoprotective signal transduction. Heart failure is a vulnerable disease state in which any additional myocardial damage may lead to acute circulatory failure. However, susceptibility to ischemic injury and the protective potential of IP in heart failure are unresolved.

Methods: We aimed to directly prove the presence of human mito-Cx43 hemi and to evaluate channel characteristics in failing versus non-failing myocardium. Furthermore we combined heart failure with ischemia-reperfusion injury in a mouse model. We used direct single-channel recordings of cardiac mitochondria to possibly identify a functional link between mito-Cx43 hemi and susceptibility to ischemic injury in murine and human heart failure.

Results: We identified single-channel currents, which were clearly distinct from mitoKATP channels, inhibited by the Cx43 mimetic peptide 43GAP27 which suppressed open probability and single-channel conductance. Single-channel properties thus supported the notion that we were recording mitochondrial Cx43 channels. Basal mito-Cx43 hemi activity was increased in both human and murine heart failure. A pharmacological preconditioning with diazoxide stimulated single-channels of failing and non-failing hearts to a similar maximal activity. Despite a reduced content and dephosphorylation of total Cx43 in failing hearts, mitochondrial Cx43 was increased and hyperphosphorylated.

Conclusion: For the first time we directly recorded human mitoCx43 hemichannels which were inhibited by 43GAP27. Our results indicate endogenous protective adaptation mediated via enhanced basal mito-Cx43 hemichannel activity through hyperphosphorylation of the mitochondrial Cx43 fraction in heart failure.

241 Glucagon-like peptide-1 receptor activation ameliorates cardiac steatosis and reverses pathological remodeling by quality control of mitochondria via cAMP/PKA axis



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Purpose: Glucagon-like peptide-1 receptor (GLP-1R) agonist exendin-4 facilitates cardiac contractility in systolic dysfunction model in rodents and patients with heart failure. However, the impact of GLP-1R on cardiac steatosis remains unclear.

Methods and Results: Male KK/Ay mice (16-week-old) were allocated into exendin-4 (24 nmole/kg/day, 40 days; KK-ex4) and vehicle group (KK-v). Male C57BL6 mice were fed with high fat diet for 3 months (DIO) and subjected to exendin-4 treatment at the age of 16 week old (DIO-ex4 and DIO-v). KK-ex4 exhibited decline in heart weight (-16.5% vs KK-v) without body weight loss. Oil-red-O staining revealed that KK-ex4 and DIO-ex4 reduced cardiac steatosis. Echocardiography revealed that systolic function of DIO-v was suppressed (-13.8% vs control), which was restored in DIO-ex4 with reduced LVPWd (-11.1% vs DIO-v) and IVSd (-30.0% vs DIO-v). Myocardial fibrosis and tissue oxidative stress detected by DHE staining were reduced in KK-ex4 and DIO-ex4 (0.45±0.10 fold and 0.58±0.10 fold vs vehicle). Transmission electron microscopy revealed restoration of irregular alignment and increase in number of mitochondria with normal cristae in KK-ex4 and DIO-ex4. Mitochondria-specific dyes (MitoTracker Red) revealed decline in oxidative activity of cardiac mitochondria both in KK-ex4 and DIO-ex4. The levels of myocardial PINK/Parkin, the surrogate indicators for damaged mitochondria, were reduced both in KK-ex4 and DIO-ex4.

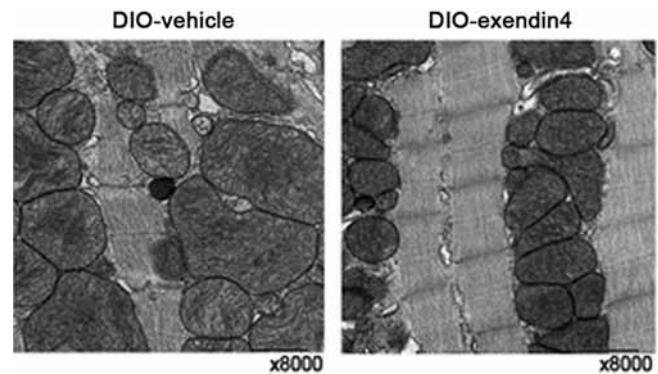


Figure 1. Typical mitochondria images by TEM

Conclusion: GLP-1R activation protects cardiac remodeling induced by steatosis through the restoring mitochondrial oxidative damage mediated by the activation of cAMP/PKA-dependent mechanisms.

242 Expression and physiological role of the novel adipokine nesfatin-1 in cardiomyocytes



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Purpose: Nesfatin-1 is a novel adipokine involved in the control of food intake and energy metabolism which shows anti-inflammatory properties. The role of this adipokine in cardiovascular physiology is unknown. In previous studies we determined that nesfatin-1 is expressed in human, rat and mouse heart. Our aim now is to study the effect of this adipokine in cardiomyocytes and the possible regulation of nesfatin-1 cardiac synthesis by diet and inflammatory mediators.

Methods: Real-time PCR was used to determine nesfatin-1 mRNA levels in cultured neonatal cardiomyocytes of Sprague Dawley rats treated with TNF-α, dexamethasone and insulin. In heart tissue of rats fed with high fat diet for 16 weeks, we used real-time PCR to determine nesfatin-1 cardiac mRNA levels and an ELISA to determine nesfatin-1 plasma levels. Cardiomyocytes were treated with nesfatin-1 and confocal microscopy was used to study the glucose transporter Glut-4 mobilization. Finally, western blot was used to identify possible transducing signalling molecules (Erk 1/2, AMPK and AKT) after nesfatin-1 treatment in cardiomyocytes.

Results: Cardiomyocytes treatment with 0.1-20 ng/ml TNF-α for 6-48 h induces an increase of nesfatin-1 mRNA levels with a maximum stimulatory effect at 20 ng/ml for 24 h (p=0.0159; Fold-Change (FC)=1.16, n=5). Treatment with 0.1-100 nM dexamethasone for 6-48 h also increases of nesfatin-1 mRNA levels with a maximum stimulatory effect at 100 nM for 24 h (p=0.0079; FC=2.457, n=5).