P77

The effect of urotensin II on calcium regulation during excitation-contraction coupling

H. Al Ali; GC. Rodrigo; DG. Lambert
University of Leicester, Cardiovascular Sciences, Leicester, United Kingdom

Funding Acknowledgements: University of Basrah/Iraq

Introduction: Heart failure is characterized by left ventricular dysfunction due to aberrant Ca2+-regulation during excitation-contraction (E-C) coupling. Plasma levels of UII is elevated in patients with heart failure, and me be related to dysfunction of Ca2+ hemostasis. The aim of this study is to investigate influence of UII on E-C coupling and Ca2+-regulation.
Methods: The Langendorff perfused isolated rat heart was used to study effect of UII on left ventricular function. Adult Rat Ventricular Myocytes (ARVMs) were isolated from Wistar rats by enzymatic digestion. Percentage cell shortening of ARVMs was measured using the video-based edge detection system. Intracellular Ca2+ was measured in single freshly isolated ARVMs loaded with Fura-2 and sarcoplasmic reticulum Ca2+ content [Ca2+]SR was determined by rapid application of 10mM caffeine. Action potential was recorded using the whole cell patch clamp technique and resistance pipette value of the seal was (12-15MΩ).
Results: Exposure of rat heart to 50nM UII Caused a 19.7 ± 2.5% reduction in left ventricular developed pressure (LVDP) (n = 8 hearts). The mechanical properties of ARVMs were assessed, the results show a significant reduction in the cell shortening from 12.6 ± 0.5% control to 10.9 ± 0.5% in UII-treated cells (n = 71 cells) (P<0.0001).
Superfusion of ARVMs with 200nM UII caused a reduction in systolic [Ca2+]i from 374.6 ± 19.9 in control to 304.7 ± 12.2 (P<0.0001) but had no effect on diastolic [Ca2+]i 54.5 ± 5.1 control and 55.9 ± 3.7 UII (n = 76 cells). Measurement of [Ca2+]SR show there was a reduction in the peak SR Ca2+ release from 440.8 ± 13.9 nM in normal Tyrode (NT) to 412.1 ± 14.7 nM in 200nM UII (n = 85 cells) (P<0.01). To determine the effects of UII on SR Ca-leak, AVRMs loaded with Fluo-3 were perfused with Ca2+ and Na+-free Tyrode (10mmol EGTA). The difference between the diastolic [Ca2+]i was measured in the presence and absence of tetracaine which blocks RyR, reflect SR Ca-leak through RyR. The data show that UII had no effect on this difference at 15.3 ± 2.6 nM in NT (n = 19 cells) and 17.1 ± 2 nM in UII (n = 25 cells) (P>0.05). To determine the effects of UII on electrical activity, the percentage change in action potential duration (APD30/50/90) was determined in response to UII (200nM). The data show APD30 was significantly reduced from 9.5 ± 1.4 ms in NT to 5.3 ± 0.9 ms in UII (P<0.01) and APD50 from 19.52 ± 2.1 ms in NT to 12.1 ± 1.6 ms in UII (n = 41 cells) (P<0.001). There was no significant change in APD90.
Conclusion: UII reduces LVDP and contraction of ARVMs. This reduction in contraction reflects a decrease in systolic [Ca2+]i, which may result from either the decline in SR Ca2+ content and/or a reduction in APD.

P78

Electrophysiological characterization of induced pluripotent stem cell-derived cardiomyocytes from duchenne muscular dystrophy patients

JM. Pioner[1]; R. Coppini[2]; L. Santini[2]; C. Palandri[2]; E. Bennati[3]; M. Regnier[4]; L. Sacconi[5]; E. Cerbai[2]; C. Poggesi[1]; C. Ferrantini[1]
[1]University of Florence, Department of Experimental and Clinical Medicine, Florence, Italy; [2]University of Florence, Department of NEUROFARBA, Florence, Italy; [3]University of Florence, AOU Meyer Children's Hospital, Florence, Italy; [4]University of Washington, Department of Bioengineering, Seattle, United States of America; [5]University of Florence, European Laboratory for Non-Linear Spectroscopy, Sesto Fiorentino, Italy

Funding Acknowledgements: Telethon grant GGP16191

Background: Cardiomyopathy invariably affects teenage patients with Duchenne Muscular Dystrophy (DMD) and account for half of the mortality. However, the molecular sequalae due to loss of dystrophin is still poorly understood.
Purpose: We analyzed time-points of electrophysiological maturation of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to provide insights into early-stage of disease mechanisms.
Methods: We combined a substrate nanopatterning approach with long-term cultures to improve maturation of hiPSC-CMs. We first studied time points of healthy hiPSC-CM maturation after 100 days in culture. To study the consequences of dystrophin loss, we used a CRISPR-Cas9 genome edited cell line targeting wild-type dystrophin locus in the control-hiPSC line (DMD-CM1). We then compared hiPSC-CMs derived from a DMD patient (DMD-CM2) with deletion of exon 50 on DMD gene causing total lack of dystrophin. We studied action potential (AP) and Ca2+ transients (Ca-T) of Control- and DMD-CMs at different pacing frequencies (0.5-1-2Hz and post rest potentiation) or with pharmacological compounds (i.e. Isoproterenol).
Results: Day 20 post-differentiated single hiPSC-CMs were plated onto nanogrooved surfaces and measured for 3 months at progressive time-points. Later stage control-CMs showed hyperpolarized resting membrane potential (RMP, -80 mV) and larger AP amplitude (110 mV) compared to the early-stage counterparts. Moreover, the AP duration (APD90) was prolonged with more resemblance to ventricular APs (600ms). Not least, later-stage control-CMs showed decreased spontaneous beating rate. In later-stages, Ca-T amplitude was almost 3-fold higher compared to early-stage CMs. Ca-T rise (time to peak, ms) became increasingly faster, while Ca-T decay (RT50) were prolonged. We evaluated the potentiation of Ca-T after a pacing pause (post rest potentiation), which was progressively increased, suggesting improved sarcoplasmic reticulum regulation of Ca2+-handling. Isoproterenol showed negligible positive inotropic and lusitropic effects and did not induce spontaneous Ca-T release in later-stage CMs, suggesting still immature β-adrenergic response.
We are investigating the electrophysiological consequences of dystrophin-null hiPSC-CM lines, which showed preserved rate of cell fractional shortening, but depressed post rest potentiation and prolonged cell relaxation (RT50) in later-stages. Both DMD-CM lines showed prolonged Ca-T decay. In addition, DMD-CM myofibril mechanics showed deficit of force, prolonged myofibril relaxation phase (slow tREL and fast kREL) and increased Ca2+-sensitivity of force development (pCa50).
Conclusion: (1) Modelling time points of DMD-hiPSC-CM maturation may predict developing disorders of DMD cardiomyopathy in vitro (2) Slower relaxation kinetics may be due to both impaired myofibril properties and Ca2+ handling abnormalities (3) Altered Ca2+ handling is a leading consequence of loss of dystrophin in hiPSC-CMs.

P79

the effects of vagus nerve stimulation on ventricular electrophysiology and nitric oxide release in the rabbit ventricle

P. Pongpaopattanakul; R. Chuahan; E. Wake; GA. Ng
University of Leicester, Cardiovascular sciences, Leicester, United Kingdom

Background: Autonomic imbalance is a known hallmark of heart failure (HF). Our group has previously shown that vagus nerve stimulation protects the heart against ventricular fibrillation (VF) via a nitric oxide (NO) pathway which could be relevant in HF. Clinical studies using vagus nerve stimulators in HF have produced ambiguous outcome results and it has been questioned whether an adequate level of stimulation was actually achieved which raises the question as to the optimal stimulation parameters needed to produce relevant functional effects
Purpose: To assess the effects of different voltages and frequencies of right VNS on cardiac electrophysiology and NO release in the rabbit ventricle.
Methods: Hearts from adult male NZW rabbits (n=20, 2.0–2.5Kg) were procured for dual-innervated Langendorff perfused preparations ex vivo. The right cervical vagus was stimulated at 2 ms with i) high voltage (80%ΔHRmax) at 1,2, and 3 Hz, and ii) low voltage (10%ΔHRbaseline) at 5, 10, and 20 Hz. Effective refractory period (ERP) and monophasic action potential duration restitution (MAPDR) were measured using a single extra-stimulus protocol. Ventricular fibrillation threshold (VFT) was measured using a burst pacing protocol (30x30 ms) as the minimum current required to induce sustained VF. NO release in the left ventricle was investigated using a single bifurcated light guide system and DAF2-DA dye. Data presented as mean±SEM, p<0.05 considered significant.
Results: Right VNS (RVNS) at a voltage that gave 80%ΔHRmax (6.71±0.67 V) reduced HR significantly at 1, 2 and 3 Hz (-13.64±2.26%, -18.29±2.42%, and -26.86±3.29% respectively). At voltages that resulted in a 10%ΔHRbaseline (1.31±0.12 V), changes in HR were less - even at 5, 10 and 20Hz (-5.39±1.60%, -6.29±1.70%, and -8.39±2.20% respectively). High levels of NO release were observed at 10 and 20 Hz of 10%ΔHRbaseline stimulation, (81.93±29.32 and 230.60±54.70 mV). RVNS increased VFT during all stimulation protocols
Conclusion: High voltage VNS affects the level of HR reduction more but not the level of NO release in the rabbit ventricle. In contrast, high frequency VNS, even at very low voltages, alters NO release whilst causing relatively little changes in HR suggesting a potential mechanistic pathway for relevant clinical application.

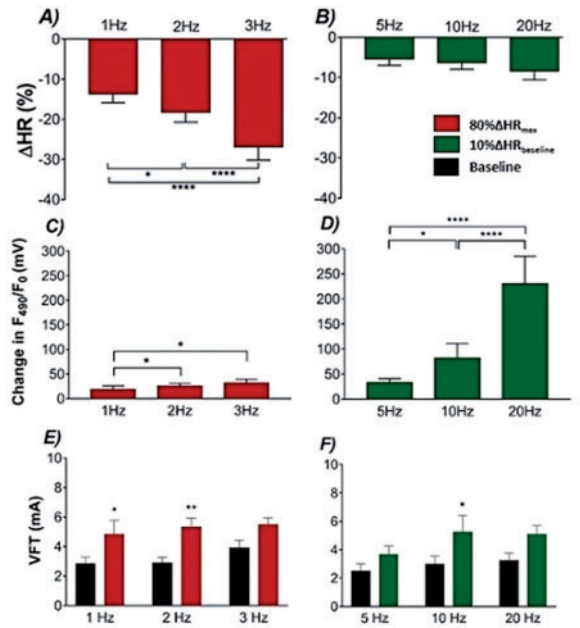

Figure 1. Percentage change of heart rate reduction (ΔHR), increased in F_{490} nitric oxide fluorescence signal, and changed in ventricular fibrillation threshold (VFT) with vagus nerve stimulations at 80%ΔHR$_{max}$ and 10%ΔHR$_{baseline}$ voltages. *P<0.05, **P<0.01, ****P<0.0001

Abstract P79 Figure.

P80

Mitochondrial dysfunction and nitric oxide levels in men with hypertension exposure of electromagnetic radiation at microwave frequencies

VS. Potaskalova[1]; MM. Seliuk[2]; MV. Khaitovych[1]; AP. Burlaka[3]; MM. Kozachok[2]; OV. Seliuk[2]
[1]National O.O. Bohomolets Medical University, Kiev, Ukraine; [2]Ukrainian military medical academy, Kiev, Ukraine; [3]R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NAS Ukraine, Kiev, Ukraine

Funding Acknowledgements: NitricOxide,ArterialHypertension, ultra-high frequency electromagnetic radiation
On behalf of: VS. Potaskalova
NO produced by nitric oxide synthase platelets, inhibits platelet aggregation and exhibits antithrombogenic effect in the vascular endothelium. EMR UHF increases the rate of generating of

superoxide radicals. It is also known contingency effects of superoxide radical and NO in the implementation of oxidative stress. The aim of the study was to determine the level of NO production of platelets in patients with AH under the influence of EMR UHF.

Materials and methods. The study included 106 men who were divided into 3 groups: Group 1 included 40 patients with hypertension at a mean age 32,5±6,1 years old, who worked under the influence of EMR UHF; Group 2 - 40 patients with AH without prolonged exposure EMR UHF; Group 3 - 26 apparently healthy men without the influence of EMR UHF (control group). EMR UHF dose for group 1 on average up to 17,151.7 ± 7102,4 kW.

The level of NO production was measured by platelet electron paramagnetic spectroscopy, the degree of oxidative damage to mitochondria was measured in terms of urinary excretion of 8-hydroxy-2-deoksiguanozina (8-OhdG).

Results: On the average in patients of Group 1 the level of NO in platelets was 2,22±1,79 cu whereas in patients of Group 2 - 0,54±0,02 cu ($P<0.05$), and men of the control group 0,95±0,16 cu. The level of urinary excretion of 8-OhdG in patients of the first group was also significantly higher than the average value of the group II patients and control group 3 (respectively 14,22±8,68 mmol/kg/day compared to 7,12±5,08 mmol/kg/day, $P<0.05$ and 0,63±0,15 mmol/kg/day, $P<0.05$.

In patients of the first group was found the highest direct correlation between the level of the daily urinary excretion of 8-OhdG and the level of nitric oxide in platelets ($r=0,39$; $P<0.05$).

Conclusions: In patients with hypertension under the effect of EMR UHF the level of nitric oxide in platelets dramatically increased and correlated with urinary markers of oxidative DNA damage, indicating that the contingency of oxidative and nitrosyl stress in the genesis of the disease.

P81

Desmin mutations impair mitochondrial function by promoting mtDNA release

N. Smolina[1]; A. Khudiakov[1]; G. Sjoberg[2]; T. Sejersen[2]; A. Kostareva[1]
[1]*Almazov National Medical Research Centre, Saint Petersburg, Russian Federation;* [2]*Karolinska Institute, Stockholm, Sweden*

Funding Acknowledgements: RSF 14-15-745П

Background: Desmin is a major intermediate filament in muscle tissue. Desmin-related myopathy (DMP) is an autosomal dominant disorder caused by mutations in desmin gene. Depending on its aggregate capacity, mutations may be subdivided into aggregate and non-aggregate. Regardless the type of mutation, DMP phenotypes have a common pathological trait—mitochondrial dysfunction. MtDNA intracellular content and extracellular release considered to be biomarkers of mitochondrial dysfunction.

Purpose: To estimate mtDNA intracellular content and extracellular release from cells harbouring desmin mutations.

Methods: Primary satellite cells were enzymatically isolated from murine soleus muscle. Cells were transduced via lentiviruses encoding desmin non-aggregate (S12F, A213V) or aggregate (L345P, A357P, L370P, D399Y) mutations, or desmin WT. When cells reached 80% of confluence, differentiation was induced by replacement 10% FCS with 2% HS. Cells and conditioned media for analysis were harvested before the differentiation and on the 3rd, 7th, and 11th day of differentiation. Conditioned media was pre-cleaned from debris by centrifugation at 300 xg for 5 min. Microvesicles were pelleted by centrifugation of the pre-cleaned media for 30 min at 16000 xg, and the supernatant was regarded as containing freely circulating mtDNA. To estimate quantity of mtDNA within the cell and in extracellular space, qPCR was performed. Absolute quantification of mtDNA was done using standard curve generated from serial dilution of mtDNA fragment of the given size simultaneously amplified at the same qPCR run.

Results: Intracellular mtDNA content didn't differ between cells harbouring miscellaneous desmin types, while mtDNA release greatly varied between cell types and between time points. All aggregate desmin mutations affected mtDNA release, however non-aggregate did not show consistent effect. In non-differentiated cells, no differences in mtDNA release were found regardless examined fraction or mutation. On the 7th day of differentiation, mtDNA release was significantly increased in microvesicular fraction in cells harbouring aggregates. On the other hand, on the 11th day of differentiation this increase dissipated. In contrast, release of freely circulating mtDNA on the 7th day was comparable between all cell types, whilst it was remarkably enhanced on the 11th day of differentiation in cells harbouring aggregates.

Conclusions: To sum up, desmin mutations impair mitochondrial function within muscle cells via intensifying mtDNA release. The effect size greatly depends on the type of mutation and on the duration of its action. Aggregate mutations as well as extended period of differentiation resulted in enhanced mtDNA release. Since freely circulating mtDNA acts as a strong pro-inflammatory trigger, enhanced mtDNA release in the presence of aggregate desmin mutations might contribute to pathogenesis of DMP, promoting inflammation process.

P82

ATM regulates cardiac mitochondrial oxidative phosphorylation potential

B. Huisamen; M. Blignaut; A. Lochner
University of Stellenbosch, Div Medical Physiology, Faculty of Medicine and Health Sciences, Stellenbosch, South Africa

Funding Acknowledgements: National Research Foundation of South Africa

Ataxia Telangiectasia (A-T) is a rare, recessive genetic disease that arises due to a decrease or absence of Ataxia Telangiectasia mutated protein kinase (ATM). A-T results in insulin resistance, type 2 diabetes and cardiovascular disease, while the absence of ATM has been associated with increased oxidative stress. ATM also maintains myocardial glucose and redox homeostasis. Using animal models, we demonstrated that myocardial ATM expression is (i) low in obesity and (ii) located on the inner mitochondrial membrane. In light of the potential importance of mitochondrial dysfunction associated with cardiovascular disease, this study aimed to investigate the role of mitochondrial ATM in cardiac oxidative phosphorylation (oxphos) using male Wistar rats.

Methods: TEM and SR-SIM microscopy and western blotting were employed to localize ATM. Ex vivo perfusion ($n=6$-9/group) of hearts ± the specific ATM inhibitor, KU60019 (3 uM) or insulin (0.3 IU/L), was performed prior to mitochondrial isolation and oxidative phosphorylation measurements (Clarke-type electrode) using either a carbohydrate (glutamate) or fatty acid (palmitoyl-carnitine) substrate.

Results: Sequential shearing off of mitochondrial membranes coupled to western blot analysis of marker proteins demonstrated the localization of ATM to the inner mitochondrial membrane. SR-SIM using fluorescently labelled antibodies confirmed this by showing co-localization with ANT (inner membrane) but not VDAC (outer membrane). Inhibition of ATM significantly decreased active (State 3) and resting (State 4) mitochondrial respiration (O2 consumption) which was associated with a decreased oxphos rate (ADP/O x QO2 State 3) and respiratory control index (RCI) ($p<0.05$) with both substrates. In contrast, stimulation of hearts with insulin, increased mitochondrial respiration parameters that, in turn, were reversed by inhibition of ATM. Moreover, inhibition of ATM resulted in decreased total mitochondrial Drp1 levels indicating less fission.

Conclusions:Taken together, this suggests that ATM plays an important role in mitochondrial oxidative phosphorylation and may be involved in mitochondrial dysfunction in insulin resistance.

P83

correlation between glycaemic status and left ventricular diastolic function in type 2 Diabetes patients

MM. Mohamed Eldeib
Al-Azhar University, cardiology, Cairo, Egypt

Background: Diabetes mellitus is considering an important independent factor in developing diastolic dysfunction. Diastolic dysfunction comprises about 30 to 50% of all patients hospitalized for heart failure. The aim of this study was to determine the correlation between glycaemic status and left ventricular diastolic function in type 2 diabetic patients

Methods: our study included (100) subjects,20 normal healthy subjects ,80 known to be Diabetic patients presented in our diabetic outpatient clinic and echocardiographic unit at an University Hospital between November 2010 and June 2011.the patient were classified according glycaemic status in to three groups: Group (A) Normal healthy control subjects. Group (B) well controlled diabetes HbA1C less than 7, Group (C) uncontrolled diabetes HbA1C more than 7.

Results: There was no statistically significant difference between the three groups as regard LVEDD,LVESD and LV EF%. There was statistically significant difference between the three groups as regard LA dimension mean E wave, mean of A wave mean of E/A ratio, mean of DT, mean of IVRT, mean of Em wave, mean of E/Em degree of diastolic dysfunction.

There was negative correlation between HbA1c level and E wave, E/A, Em and positive correlation with LA dimension, A wave, IVRT, DT and E/Em.

conclusion: The Glycemic status is well correlated with severity of diastolic dysfunction in asymptomatic type 2 diabetic patients. Tissue Doppler imaging has been shown to be more sensitive and more independent for assessment of diastolic function in asymptomatic type 2 diabetic patients and its results are significant correlated with glycemic state.

P84

Metabolomic profiling of human fetal cardiac mesenchymal stromal cells

I. Bulatovic[1]; C. Folmes[2]; I. Vuckovic[2]; S. Zhang[2]; C. Osterholm[1]; A. Terzic[2]; KH. Grinnemo[1]
[1]*Karolinska Institute, Department of Molecular Medicine & Surgery, Division of Cardiothoracic Surgery, Stockholm, Sweden;* [2]*Mayo Clinic, Division of Cardiovascular Disease, Rochester, United States of America*

Funding Acknowledgements: Karolinska Institute-Mayo Clinic Collaborative Grant 2013

On behalf of: Heartlab, Karolinska Institutet

Mesenchymal stromal cells (MSCs) are an attractive source for cell therapy and tissue engineering applications due to their multipotency and immunomodulatory capacity. However, the full therapeutic potential of MSCs is not yet achieved because of their premature proliferative senescence, incomplete differentiation to desired cell types and poor engraftment in injured tissue. The critical role of metabolic pathways in the active control of cell renewal and lineage specification has recently come into focus, but signaling pathways connecting energy metabolism and cell fate decision are still to be elucidated. Wnt-signaling controls many biological processes including self-renewal and differentiation of cardiac progenitors. Moreover, regulation of the cellular metabolism may represent a general mechanism contributing to the wide-range of functions controlled by Wnt-proteins.

In this study, we have characterized the bioenergetic functions of human fetal cardiac MSCs (hfcMSCs). Oxygen consumption rate and intracellular ATP content were increased in MSCs during fetal development, indicating an increase in aerobic mitochondrial metabolism. Interestingly, in hfcMSCs derived from a 9-week fetal heart, a sustained stimulation of WNT3A signaling during the expansion, led to further activation of oxidative phosphorylation (OxPhos). Stimulation of the WNT/β-catenin signaling pathway under hypoxia appeared to enhance long-term MSC expansion, and resulted in a cell population with higher cardiogenic differentiation potential.

In summary, our work provides important insights in MSC bioenergetics and proposes a WNTs role in regulation of mitochondrial OxPhos in these cells. The interplay of energy metabolism with stem cell fate decisions opens a new perspective in understanding developmental principles and offers a potential target for controlling tissue homeostasis and regeneration. Manipulation of metabolic signals may thus facilitate endogenous stem cells activation, tissue renewal or adoption of cytoprotective behavior.

P85

Combined transcriptomics, proteomics and metabolomics analysis identifies metabolic pathways associated with the loss of cardiac regeneration

V. Talman[1]; J. Teppo[2]; P. Poho[1]; P. Movahedi[3]; A. Vaikkinen[1]; T. Pahikkala[3]; T. Kotiaho[4]; R. Kostiainen[1]; M. Varjosalo[5]; H. Ruskoaho[1]
[1]*University of Helsinki, Drug Research Program, Faculty of Pharmacy, Helsinki, Finland;* [2]*University of Helsinki, Drug Research Program, Faculty of Pharmacy and Institute of Biotechnology, Helsinki, Finland;* [3]*University of Turku, Department of Future Technologies, Faculty of Mathematics and Natural Sciences, Turku, Finland;* [4]*University of Helsinki, Drug Research Program, Faculty of Pharmacy and Department of Chemistry, Helsinki, Finland;* [5]*University of Helsinki, Institute of Biotechnology, Helsinki, Finland*

Funding Acknowledgements: Tekes (project 40395/13), Finnish Foundation for Cardiovasc. Research, Sigrid Juselius Foundation