Background: The insufficient regenerative capacity of the adult human heart represents a great therapeutic challenge, and treatments that could promote regeneration are urgently needed. The mammalian hearts lose their intrinsic regenerative capacity within the first week of postnatal life. This coincides with a metabolic switch in cardiomyocytes, in which the energy production converts from glycolysis to lipid oxidation. More detailed understanding of the metabolic changes and regulation of cardiomyocyte cell cycle withdrawal is required for the development of regenerative therapies.
Purpose: The aim of this work was to elucidate the metabolic changes occurring in the murine heart in the early postnatal period in order to identify potential new drug targets for the development of cardiac regeneration-inducing therapies.
Methods: Ventricular tissue samples from C57/Bl6 mouse pups at postnatal days 1, 4, 9 and 23 (P1, P4, P9 and P23, respectively) were analysed using genome-wide RNA sequencing, quantitative shotgun proteomics (LC-MS/MS) and global metabolomics (LC-MS, GCxGC-MS). RNA sequencing was performed from one set of tissue samples (Set 2, n=3 pooled samples per group, each from 3 hearts) while proteomics and metabolomics analyses were carried out with two separate sets of tissue samples (Sets 1 and 2; individual hearts analysed separately, n=5-15 for each group in both sets). Differential expression analysis was carried out and changes were considered significant when FDR-corrected $p<0.01$. For RNA sequencing and metabolomics, a fold change limit of 1.5 was also applied. Fuzzy clustering, gene set enrichment analysis and KEGG pathway analysis were performed to identify correlative changes in RNA, protein and metabolite data.
Results: The multiomics analysis identifies a number of metabolic pathways with significant and correlative changes on all levels (mRNA, protein, metabolites) within the early postnatal period. These include previously reported changes in energy metabolism, down-regulation of glycolytic pathways and up-regulation of fatty acid oxidation, as well as metabolic pathways, whose role in cardiac maturation and regeneration has not been reported. Several examples of interesting metabolic pathways that are time-wise associated with the postnatal loss of regenerative capacity of the murine heart will be presented. These may represent attractive therapeutic targets for heart failure.
Conclusions: Combining RNA sequencing with proteomics and metabolomics provides a powerful tool for understanding changes in metabolic pathways and for the identification of novel drug targets. The present study provides the first resource of combined transcriptomics, proteomics and metabolomics data from the early postnatal hearts associated with the loss of regenerative potential.

P86

Stimulation of endogenous glutathione synthesis prevent postreperfusion NOS uncoupling, oxidative nitrosative stress and cardiodynamic disturbances in rats

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Ischemia-reperfusion of isolated rat hearts is accompanied by cNOS uncoupling (index of cNOS coupling decreased 20-times), oxidative stress (generation rate of superoxide radical was increased 3-times), decrease of cNOS activity, development of contracture and myocardial contractility insufficiency. Adult Wistar rat hearts were isolated by Langendorff preparation underwent a 20-minute ischemia and a 10-minute reperfusion before biochemical examination for superoxide radical, hydroxyl radical, NOS activity and glutathione content. Pre-treatment with L-cysteine (120 mg/kg), precursor of glutathione and H2S synthesis, in combination with inhibitor of H2S synthesize enzyme CSE – PAG (11 mg/kg) increased 3-times glutathione level. GSH consumption after ischemia-reperfusion increased 4-times and its conversation to oxidized form (GSSG) increased 6-times in comparing to control reaction. It was accompanied by significantly better redox status of myocardial tissue and cardiodynamic disturbances prevention. Inhibition of GSH synthesis by BSO (buthionine sulfoximine 22 mg/kg) restored above disturbances. Thus, glutathione levels in cardiac tissue predetermine heart reaction in ischemia-reperfusion and stimulation of GSH synthesis prevents typical disturbances for this reaction.

P87

Deleterious effects of FAAH-deficiency on development of ischemic cardiomyopathy in mice

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Introduction: Ischemic cardiomyopathy is associated with repetitive ischemia and reperfusion (I/R), which leads to inflammatory reaction and left ventricular (LV) dysfunction. Animal studies provided evidence for cardioprotective effects of the endocannabinoid system after myocardial ischemia including modulation of cardiomyocyte adaptation, inflammation and remodeling. Endocannabinoid receptor CB2-deficiency led to increased cardiomyocyte apoptosis and infarct size, accompanied by worsened LV function. Otherwise, endocannabinoids can act as Peroxisome proliferator-activated receptor (PPAR)-α agonist, and its activation causes lipotoxicity leading to cardiomyocyte apoptosis.
Purpose: Therefore, we investigated the impact of elevated level of endocannabinoid anandamide in fatty acid amide hydrolase (FAAH)-/- mice undergoing repetitive I/R.
Methods: Repetitive daily 15 min. left anterior descending artery occlusion was repeated over 1, 3 and 7 d in C57/Bl6 (WT) and FAAH-/- mice (n≥8). PPAR-α mediated effects of high anandamide levels in FAAH-/- mice were eliminated with selective PPAR-α antagonist GW6471 i.v. Proof of principle was done by blocking the effect of agonist WY14,634 on PPAR-α downstream gene-upregulation with antagonist GW6471 in WT. LV function was assessed using M-mode echocardiography. Immunohistochemical analysis revealed collagen deposition (Picrosirius red), macrophage accumulation (MAC-2) and remodeling (αSMAC). Hypertrophy was determined by cardiomyocyte area and heart weight/tibia length. Molecular analyses involved Taqman® RT-qPCR and ELISA.
Results: FAAH-/- mice showed cardiomyocyte loss after 7 d I/R, accompanied by collagen deposition and scar formation with persistent LV dysfunction 60 d after discontinuation of I/R, while WT mice functionally and morphologically recovered after 60 d. Collagen deposition was reduced to WT-levels when FAAH-/- mice were treated with PPAR-α antagonist GW6471. Expression of chemokine CCL2 was significantly higher in FAAH-/- mice and accompanied by higher macrophage infiltration in areas of cardiomyocyte loss, which was also attenuated by GW6471 and comparable to WT. Significantly reduced induction of cardioprotective antioxidative enzyme HMOX-1 and energetically more efficient myosin heavy chain isoform β-MHC in FAAH-/- mice was normalized to WT-level by GW6471. Further, hypertrophy and adverse remodeling with high myofibroblasts accumulation observed in FAAH-/- mice was diminished by PPAR-α antagonism.
Discussion: Our study gives novel insights in the role of endocannabinoids acting – at least in part – via PPAR-α in murine ischemic heart. We hypothesize that uncontrolled increase in endocannabinoids may have detrimental effects on cardiomyocyte survival due to PPAR-α activation with lipotoxicity and subsequent increase in inflammatory response.

P88

High molecular weight adiponectin concentration and body mass index as prognostic markers of atrial fibrillation development in the general population

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Leptin and adiponectin are both closely related with obesity and have recently been implicated with AF. These adipokines have been proposed as links between adiposity and insulin resistance, glucose deregulation, and CVD.
Objective: Evaluate the probability of developing atrial fibrillation (AF) depending of BMI and levels of high molecular weight adiponectin (HMVAN)) in the general population.
Material and methods. By random sampling were examined 420 patients (192 women - 45.7%) aged 37-56 years. Availability phenotype MHO assessed according Wildman criteria: the presence of 0-1 factors indicating metabolic health (SBP ≥130 or diastolic blood pressure ≥85 mm Hg or antihypertensive therapy; triglycerides ≥1.70 mmol / l, HDL <1.04 (men) <1.30 (females) mg / dL or lipid-lowering therapy; glucose ≥5.55 mmol / l or hypoglycemic therapy, C-reactive protein (CRP)> 4.72 mg / l; HOMA-IR> 4,81). Leptin and HMVAN level were determined by ELISA (DRG, USA).
Results: Obesity (BMI ≥ 30 kg / m^2) was detected in 105 (25.0%) of participants - 1 group, BMI 25-29.9 kg / m^2 - in 186 (44.3%) patients - group 2; BMI 20-24,9 kg / m^2 - in 96 (22.9%) patients - group 3; BMI <19.9 kg / m^2 - in 33 (7.8%) patients - group 4. All datas for the MHO phenotype evaluation were available in 97 participants. According to the Wildman criteria phenotype MHO was detected in 20 (19.1%) patients, that was comparable with the data of the world's population-based studies. During follow-up (3,8 ± 1,2 years) in 33.5% of participants developed AF. Adiponectin level was significantly higher in MHO patients compared to metabolically unhealthy patients with AO (11.86 ug / ml vs 7,85 mcg / ml), $p<0.01$). HMVAN level was significantly decreased in patients 1 and 4 groups compared to groups 2 and 3. Correlation between AF and HMWAN was determined by regressive analysis in patients of 1st and 4th groups ($\beta = -0.24$, $P = 0.003$ and $\beta = -0.26$, $P = 0.002$, respectively).
Conclusions: The probability of developing AF increases with AO and decreased BMI, which is accompanied by a change in HMVAN level. In MHO patients probability of AF developing is identical with persons having normal BMI. Adipocytokine imbalance, with a low level of adiponectin, can act as a triggering mechanism for the development of atrial fibrillation.

P89

Comparative analysis of the status of the nitric oxide system in the left ventricle of heart in rats with experimental hypertension of different origin

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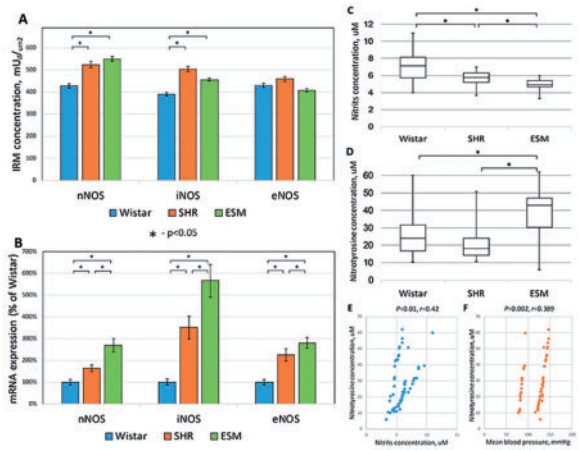
Background: The hypertension development is accompanied by endothelial dysfunction. NO and its synthases (NOS) play a key role in this event. It is assumed, that neuronal isoform (nNOS) fulfills protective role providing vasodilatation, and inducible isoform (iNOS) aggravates endothelial dysfunction via nitrosative stress, whilst endothelial isoform (eNOS) may provide vasodilatation as well as produce an excessive amount of peroxynitrite. We believe that similar changes may occur in myocardium.
The aim of study was to assess the status of the nitric oxide system in left ventricle myocardium in rats with experimental hypertension.
Methods: study was carried out in 3 groups of male rats in age of 7-8 month and weight of 220-290 grams. 1) 10 Wistar rats (mean blood pressure (mBP) 83.8±0.96 mm Hg); 2) 10 SHR (mBP 125.8±1.12 mm Hg); 3) 10 Wistar rats underwent the endocrine-saline modelling (ESM) of hypertension, as described earlier (mBP 137.8±1.23 mm Hg). Study followed the "Principles of laboratory animal care" and approved by local Commission on Bioethics.
The NOS isoforms expression was evaluated with immunofluorescent assay (IF) in paraffin-embedded myocardium slices. In left ventricle myocardium homogenates, we also assessed the mRNA expression to NOS isoforms using real-time polymerase chain reaction (RT-PCR) and the nitrites concentration (Ns) as the end reaction products of the NOS activity using biochemical assay. Moreover, we evaluated the plasma nitrotyrosine (NT) levels using ELISA with aim to assess its potential also as a biomarker.
Statistical analysis was performed using one-way ANOVA with post hoc Bonferroni correction or Kruskal-Wallis criterion with post hoc Dunn correction, when appropriate. Statistically significant differences were considered when $p<0.05$
Results: are showed at the figure 1. Data presented as M±m (A,B), or as median, 1st and 3rd quartiles, min and max (C,D), or correlation dot plots (E,F).

The multifactorial linear regression model (aR^2=0.559, p<0.001) indicated Ns (B=6.94±0.92, β=0.72; p<0.001) and mBP (B=0.417±0.057, β=0.696; p=0.001) as independent predictors of the NT plasma level in rats.

Conclusions: 1) The blood pressure elevation in rats is accompanied by increased expression both of nNOS and iNOS, whereas the eNOS level remained unchanged. 2) The changes stated above are accompanied by increased expression of mRNA of all NOS isoforms, including eNOS, which may be an evidence of the eNOS high demand in myocardium. 3) Considering the progressive decrease of the nitrates concentration in myocardium homogenates and the simultaneous plasma nitrotyrosine level increase, which are correspond to the mean blood pressure elevation, all the stated above may be considered as development of changes in myocardium, which are identical to endothelial dysfunction, including eNOS uncoupling with the shift of its activity to peroxynitrite production despite of NO.



Abstract P89 Figure.

P90

Features of the BNP and beta-endorphin expression in hypertension in different models

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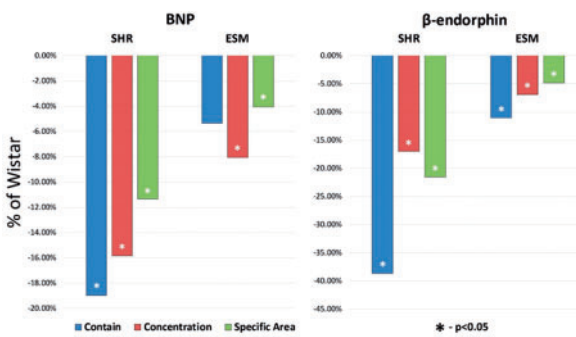
Background: The blood pressure regulation involves a complex integration of different systems comprised of 2 domains – the peripheral and the central ones. One of the main linkage structures of them is hypothalamus. This effect implements via local synthesis or provision to the regulatory niclei the complex of neurohormones which mediate pressor and depressor effects. It is considered, the pathogenesis of hypertension (HT) includes the high activity of peripheral pressor neuropeptides and/or low activity of depressor ones, but the data according to the central domain is lacking. One of the key intrahypothalamic coordinator is arcuate nucleus (Arc). We postulate that violation of the depressor neuropeptides' level in it leads to HT.

The purpose of our study was to find out the features of brain natriuretic peptide (BNP) and β-endorphin expression in models of primary and secondary hypertension in rats.

Materials and methods. Study was conducted in 3 groups of mature male rats (of 7-8 month of age and 220-270 grams of weight): 1) control group – 10 Wistar rats (mean blood pressure (mBP) 83,8±0,96 mm Hg); 2) 10 SHR (mBP 125,8±1,12 mm Hg); 3) 10 Wistar rats with endocrine-saline model (30-day intraperitoneal administration of prednisolone at 7 o'clock in dose of 2 mg/kg and at 20 o'clock in dose of 4 mg/kg with simultaneous force watering with 5 ml of 2,3% saline, mBP 125,8±1,12 mm Hg). We studied the β-endorphine and BNP in 14 um paraffin-embedded slices of hypothalamus using immunofluorescence assays. The image analysis was done in ImageJ. Statistical analysis was done with one-way ANOVA with post hoc Bonferroni correction. Significant difference was consider when p<0.05

Conclusions: Development of HT leads to decrease of level and allocation both of studied depressor neuropeptides in the structure of Arc. Their balance change depends on etiology of hypertension. The most distinct difference was observed in SHR group. We believe it is due to the exhaustion of depressor system as a result of extended blood pressure elevation.



Abstract P90 Figure.

P91

Effect of xanthine oxidase inhibition with allopurinol on autonomic regulation of the heart rhythm in normoxia and hypoxia

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Introduction: Xanthine oxidase (XO) which catalyses superoxide formation is among the major sources of reactive oxygen species (ROS) in cardiovascular system. Increased ROS level, particularly to occur during ischemia may negatively affect autonomic nervous system (ANS) control of the heart. Recent data have indicated that XO inhibitor, allopurinol (ALLO) prevents hypoxic injury of the heart.

Aim: We investigated how XO inhibition influences the ANS regulation of heart rhythm in baseline normobaric normoxia and hypobaric hypoxia in rats.

Methods: The study was approved by the Local Ethics Committee. Time-series of 1024-RR-intervals (RRi) were extracted from 4 kHz ECG recorded in conscious unrestrained Wistar rats (N=8, 300 g) in standard atmospheric conditions (normoxia), followed by 1-hour controlled hypobaric hypoxia (-400mmHg). The procedure was performed before and after ALLO administration (5 mg/kg) in two consecutive days. ANS regulation was assessed by heart rate variability (HRV) analysis (Kubios HRV Pro software) performed in time- and frequency-domains. Frequency ranges: 0.27 to 0.75 Hz (low frequency, LF) and 0.75 to 2.5 Hz (high frequency, HF) were selected for spectral powers. Nonlinear dynamics of HRV was also analysed through Sample, Shannon and Approximate entropies (SampEn, ShanEn, ApEn).

Results: Hypoxia resulted in a significant reduction of HR (from 334±9 to 288±11 BPM; p=0.003). The overall HRV, assessed by SDNN or total spectral power (TSP) did not significantly change but indexes of vagal activity were significantly increased: rMSSD (from 3.29±0.42 to 5.39±0.55ms2; p=0.02) and HF (from 3.52±1.12 to 6.37±1.02ms2; p=0.02). SampEn, ShanEn, ApEn remained unchanged. ALLO evoked a significant change of ShanEn (from 3.7±0.13 to 3.93±0.09 p=0.047) and ApEn (from 1.37±0.03 to 1.29±0.05; p=0.029) in normoxic conditions, while in following hypoxia all those changes returned to baseline. In normoxia, ALLO did not significantly changed the time-domain (SDNN, rMSSD) and spectral HRV parameters (LF, HF), but in hypoxia, ALLO evoked an increase of SDNN (from 6.08±1.05 to 11.36±2.14ms; p=0.013), TSP (from 29.94±6.57 to 138.34±36.75ms2;p=0.016) and LF (from 5.75±1.73 to 38.56±12.05ms2; p=0.02) together with parasympathetic drive (rMSSD from 5.39±0.55 to 9.33±1.59ms; p=0.019 and HF from 6.37±1.02 to 20.31±4.67ms2; p=0.014). Summary: The major finding is that XO inhibition influenced autonomic control upon the heart, especially during hypoxia when XO is supposed to increase. Changes in ShanEn and ApEn evoked by ALLO indicate the reduction of complexity of heart rate regulation. What is interesting complexity and predictability was changed without altering the sympatho-vagal balance, as shown by linear parameters. In hypoxia allopurinol resulted in a significantly increased overall autonomic control including an increased vagal drive.

P92

Reduced myofilament calcium-sensitivity in diabetic human left ventricular cardiomyocytes

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Background: The contractility of the diabetic heart is known to be diminished, but the mechanisms for this reduced contractility are not understood. We have found that cardiac β-adrenergic activation is unaffected by diabetes, and intracellular calcium regulating proteins (RyR, SERCA) are upregulated in human diabetic heart tissue, suggesting a mechanism other than intracellular calcium dysregulation compromises the contractility of the diabetic cardiomyocyte.

Purpose: This study determined whether the calcium-sensitivity of myofilament contraction was reduced in skinned human left ventricular cardiomyocytes.

Methods: After informed consent, left ventricular biopsies were obtained from open chest surgical patients who were categorized as non-diabetic with ejection fraction >50% (nDM-pEF; n = 5), non-diabetic with ejection fraction < 50% (nDM-rEF; n = 4) and diabetic with ejection fraction > 50% (DM-pEF; n = 4). Biopsied ventricular tissue was flash frozen in liquid N2 and stored at -80oC. For contractile measurements, single myocyte-sized preparations were obtained by mechanical disruption of this tissue in a Waring blender. The resulting suspension of intact myocytes, groups of myocytes and cell fragments was permeablized using 0.3% ultrapure Triton X-100. Permeablized myocyte preparations were attached between a capacitance-gauge transducer and a direct-current torque motor. Force (tension) was measured as a function of pCa (−log[Ca2+]) in the range of 9.0 to 4.5. For each activation steady force was allowed to develop, after which the cell was slackened and subsequently transferred to relaxing solution. Total force was measured as the difference between steady developed force and the baseline force immediately after the slack step. Active force was calculated by subtracting resting tension at pCa 9.0 from total force. Force at each pCa was expressed as a fraction of the maximum force (relative tension; measured in solution with pCa 4.5) obtained for that cell under the same conditions. PCa50, the calcium concentration at which 50% of maximal force was developed, was used to compare the calcium sensitivity of the two groups.

Results: A total of 44 skinned cardiomyocytes preparations were tested (nDM-pEF =16, nDM-rEF = 13, DM-pEF = 15). pCa50 was lower (p < 0.05) in DM-pEF (5.71 ± 0.04) vs. nDM-pEF (5.94 ± 0.04) and nDM-rEF (5.84 ± 0.07) groups when compared as a mean value for each patient . When all cells were compared (not individual mean values) pCa50 in nDM-pEF (5.94 ± 0.06) > nDM-rEF (5.85 ± 0.03) > DM-pEF (5.71 ± 0.06).

Conclusions: Myofilament calcium sensitivity was lower in left ventricular cells from patients with diabetes compared to non-diabetic groups. This may explain why contractility is reduced in these patients despite having normal β-adrenergic responsiveness and up-regulated cardiomyocytes calcium handling proteins.